

Exhibit 1

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

University of Western Australia,
Junior Party
(Patent 8,455,636,
Inventors: Stephen Donald Wilton, Sue Fletcher and Graham McClorey)
v.

Academisch Ziekenhuis Leiden,
Senior Party
(Application 11/233,495,
Inventors: Garrit-Jan Boudewijn van Ommen, Judith Christina Theodora van Deutekom,
Johannes Theodorus den Dunnen and Annemieke Aartsma-Rus).

University of Western Australia,
Junior Party
(Patents 7,960,541 and 7,807,816,
Inventors: Stephen Donald Wilton, Sue Fletcher and Graham McClorey)
v.

Academisch Ziekenhuis Leiden,
Senior Party
(Application 13/550,210,
Inventor: Judith Christina Theodora van Deutekom).

University of Western Australia,
Junior Party
(Patent 8,486,907,
Inventors: Stephen Donald Wilton, Sue Fletcher and Graham McClorey)
v.

Academisch Ziekenhuis Leiden,
Senior Party
(Application 14/198,992,
Inventor: Judith Christina Theodora van Deutekom).

Patent Interference Nos. 106,007, 106,008, 106,113 (RES)
(Technology Center 1600)

DECLARATION OF MATTHEW J. A. WOOD, M.D., D. PHIL.

UWA EXHIBIT 2081
University of Western Australia
v.
Academisch Ziekenhuis Leiden
Interference Nos. 106,007, 106,008 & 106,013

NS00143268

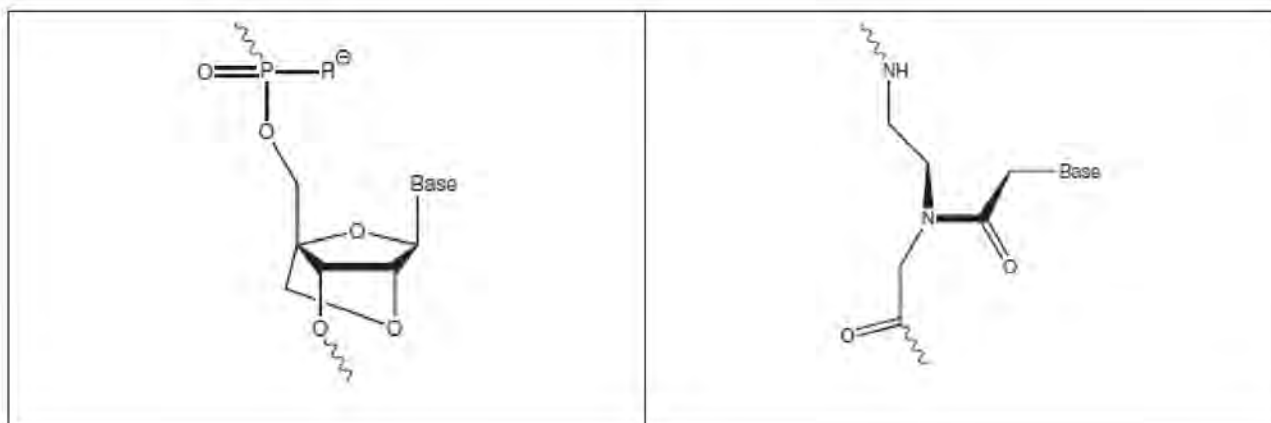
(Exh. 2005 at 173.)

2. Prominent classes of AON proposed for exon skipping

51. Because chemists can make changes at almost every position of the nucleobase, chemical backbone, and internucleotide linkages, the number of possible modifications to naturally occurring AONs is immense. In an effort to cope with the many requirements for exon skipping, scientists have explored many different AON chemistries, including AONs with modifications to the nucleobase, the backbone, the internucleotide linkages, and combinations of each.

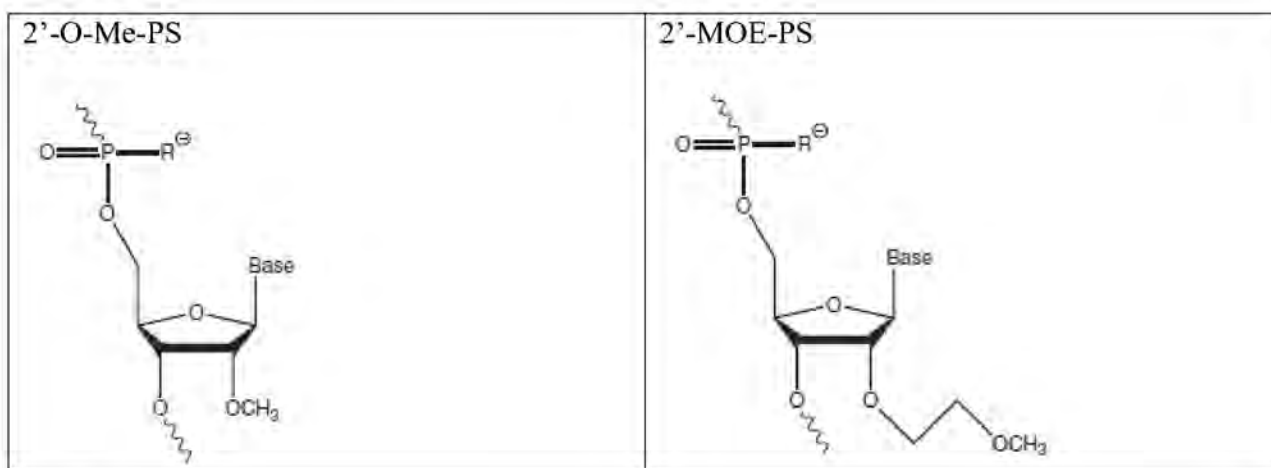
52. AON chemistries can vary significantly from naturally occurring nucleotides. However, they preserve the ability to form Watson-Crick base pairs with pre-mRNA through the maintenance of nucleobases (sometimes modified) in the correct spatial conformation. Although not exhaustive, a selection of significant types of AON chemistries proposed for exon skipping is illustrated in the following figure and discussed below. These include phosphorothioates (“PS”), morpholinos (“PMOs”), locked nucleic acids (“LNAs”), and peptide nucleic acids (“PNAs”).

<p>Phosphorothioate (PS)</p>	<p>Morpholino (PMO)</p>
<p>Locked Nucleic Acid (LNA)</p>	<p>Peptide Nucleic Acid (PNA)</p>



(Exh. 2061 at 38.)

53. Phosphorothioates are chemically similar to RNA, but the non-bridging oxygen atom of the phosphate group of RNA is replaced by a sulfur atom. As illustrated in the following figure, commonly used phosphorothioates include 2'-O-methyl phosphorothioate ("2'-O-Me-PS"), which have a methyl group attached to the oxygen atom at the 2' position of the ribose ring, as well as 2'-O-methoxyethyl phosphorothioate ("2'-MOE-PS"), which add a methoxy group instead of a methyl group. These modifications confer some nuclease resistance.



(Exh. 2061 at 38.)

54. Phosphorothioates retain a negative charge. As explained below, this property aids in their solubility, but it also makes them "sticky," meaning that they have a tendency to

nonspecifically bind proteins, which can prove problematic for efficient delivery *in vivo* and lead to toxicity.

55. Phosphorodiamidate morpholino oligomers (called “morpholinos” or “PMOs”) have six-membered morpholine rings in place of ribose. Additionally, each “nucleotide” is joined together by phosphorodiamidate linkages rather than phosphodiester linkages.

56. Morpholinos are nuclease and RNase H resistant and have no charge on their backbone at physiologic pH. Morpholinos are not toxic and are very stable, as the majority of administered compound is excreted essentially unchanged in urine. Unlike phosphorothioates, they have no net electrical charge and therefore do not tend to interact with non-target molecules. (Exh. 2005 at 174.)

57. Peptide nucleic acids (“PNAs”) replace the sugar group of DNA and RNA with repeating N-(2-aminoethyl)-glycine units linked by peptide bonds (which are typically found in proteins, not nucleic acids).

58. Like morpholinos, PNAs are uncharged at physiologic pH. PNAs are also resistant to enzyme degradation. PNAs tend to have very high target binding affinity, but PNA/RNA mismatches are more destabilizing than a similar mismatch in an RNA/RNA duplex. Unmodified PNAs cannot readily cross cell membranes, and PNAs are less soluble than RNA oligonucleotides. (Exh. 2006 at 4508.)

59. As compared to RNA, locked nucleic acids (“LNAs”) contain a bond connecting the 2'-oxygen of the ribose with the 4'-carbon. This bond “locks” the sugar portion of the nucleotide in a particular confirmation, and as a consequence LNAs are conformationally inflexible. (Exh. 2009 at 1.)

60. LNAs have an extremely high affinity for RNA and DNA. Additionally, LNAs are considered non-toxic and are RNase H resistant. However, full-length LNAs generate an effect mainly in liver, colon, and small intestine after systemic delivery, and therefore there may be issues with delivery to muscle.

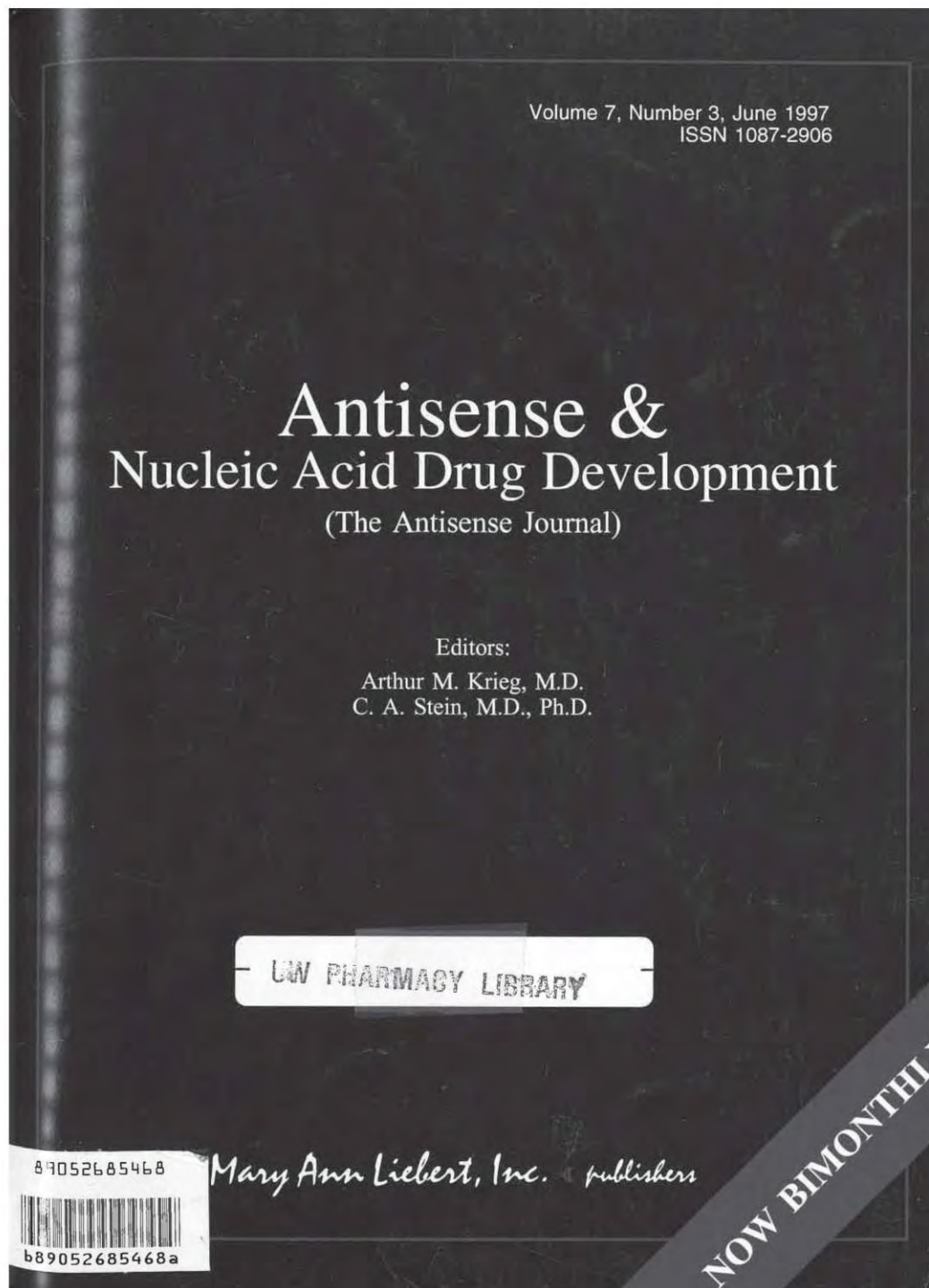
61. Many modifications have been and can be made within these commonly used frameworks. For example, non-natural modified nucleobases could be used in place of A, C, G, T, and U. Such well-known nucleobases include, by way of example only, 5-substituted pyrimidines; 6-aza-pyrimidines; and N-2, N-6, and O-6 substituted purines.

62. Such non-natural nucleobases may bind more or less tightly than their natural equivalent, and therefore may alter the affinity of a particular AON for its target. For example, 5-methyl-cytosine increases duplex stability as compared to unmodified cytosine (C). (Exh. 2007 at 3613.)

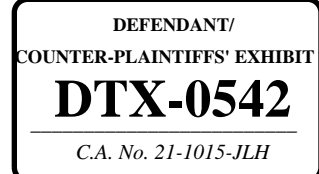
63. Researchers have also investigated AONs made with internucleotide linkages other than those called out above. These include, by way of example only, phosphotriester, methylphosphonate, phosphoroamidate, carbonyl, and sulfonyl linkages. (Exh. 2008 at 189.) Changing these internucleotide linkages can alter binding specificity and affinity, nuclease resistance, delivery to target tissue, uptake into cells, and intracellular targeting.

64. AONs can also be made that are synthesized partly with one chemistry and partly from another. These are called “chimeric” or “hybrid” AONs. For example, “gapmers” have been studied that contain a central region of 2'-O-Me-PS nucleotides flanked by LNAs. (Exh. 2006 at 4509.) Similarly, LNA/2'-O-Me RNA chimeras have been developed to improve the potency and specificity of their action. (Exh. 2006 at 4509.)

Exhibit 2



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GENERAL INFORMATION

Antisense & Nucleic Acid Drug Development, a bimonthly journal, discusses human-made substances and their effects on gene expression at the RNA and DNA levels. It provides a forum for basic researchers in molecular and cell biology, chemical synthesis, and applied therapeutics to discuss the development of new concepts and experimental approaches to understand and modulate gene activity.

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Review Article

Morpholino Antisense Oligomers: Design, Preparation, and Properties

JAMES SUMMERTON and DWIGHT WELLER

ABSTRACT

Antisense promised major advances in treating a broad range of intractable diseases, but in recent years progress has been stymied by technical problems, most notably inadequate specificity, ineffective delivery into the proper subcellular compartment, and unpredictable activity within cells. Herein is an overview of the design, preparation, and properties of Morpholino oligos, a novel antisense structural type that solves the sequence specificity problem and provides high and predictable activity in cells. Morpholino oligos also exhibit little or no nonantisense activity, afford good water solubility, are immune to nucleases, and are designed to have low production costs.

INTRODUCTION

OLIGONUCLEOTIDES, OLIGONUCLEOTIDE ANALOGS, and other sequence-specific binding polymers designed to block translation of selected messenger RNAs (the sense strand) are commonly called "antisense oligos." Development of such oligos for therapeutic applications, which constitutes the epitome of rational drug design, entails selecting a target genetic sequence unique and critical to the pathogen or pathogenic state one wishes to treat. One then assembles an oligomer of genetic bases (adenine, cytosine, guanine, and thymine or uracil) complementary to that selected sequence. When such an antisense oligo binds to its targeted disease-causing sequence, it can inactivate that target and thereby alleviate the disease.

Antisense oligos offer the prospect of safe and effective therapeutics for a broad range of intractable diseases. Nonetheless, developing therapeutics that function by a true antisense mechanism presents a number of forbidding challenges. The oligos should achieve adequate efficacy at a concentration attainable within the cells of the patient. They should inhibit their selected target sequences without concomitant attack on any other sequences in the patient's pool of approximately 200 million bases of unique-sequence RNA. They should be stable in extracellular compartments and within cells. They must be deliverable into the cellular compartment(s) containing their targeted sequences. They should be adequately soluble in aqueous solution. They should exhibit little or no toxicity at therapeutic concentrations. Finally, they should be affordable, reflecting the in-

creasing awareness that health care, even for life-threatening conditions, should not expend an excessive portion of society's resources.

First-generation antisense oligos comprised natural genetic material (Belikova et al., 1967; Zamecnik and Stephenson, 1978; Summerton, 1979) and often contained crosslinking agents for binding their targets irreversibly (Summerton and Bartlett, 1978a,b). As the design challenges became more fully appreciated, a number of nonnatural antisense structural types (Fig. 1) were developed in an effort to improve efficacy, stability, and delivery. Of particular note are the early non-ionic DNA analogs developed by Miller and Ts'o, including phosphotriester-linked DNA (Miller, 1989a) and methylphosphonate-linked DNA (Miller, 1989b). Other nucleic acid analogs of note include carbamate-linked DNA (Stirchak et al., 1987), phosphorothioate-linked DNA (Stein and Cohen, 1989), phosphoramidate-linked DNA (Froehler et al., 1988), α -DNA (Rayner et al., 1989), and 2'-O-methyl RNA (Shibahara et al., 1989). Figure 1B shows several novel antisense types that no longer resemble nucleic acids. These oligos contain acyclic backbone moieties, including nylon (Weller et al. 1991; Huang et al., 1991), the exceptionally high-affinity peptide nucleic acids (PNAs) (Egholm et al., 1992), and related types (Summerton and Weller, 1993a).

Although each of these newer structural types provides one or more significant advantages over the first-generation oligos, none yet appear to provide the full combination of properties needed in antisense therapeutics for clinical applications.

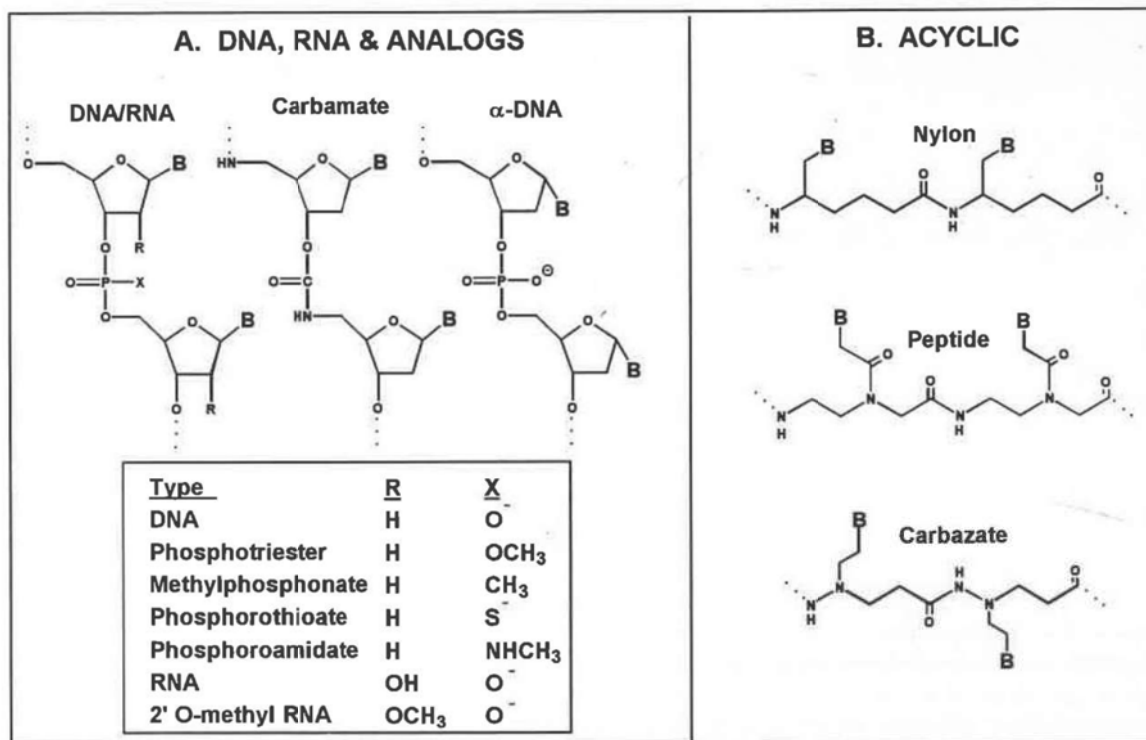


FIG. 1. Representative antisense structural types.

Herein we describe the design considerations used in developing a novel Morpholino structural type (Fig. 2), which affords antisense oligos having very high efficacy and specificity, im-

munity to nucleases, good aqueous solubility, and low production costs.

DESIGN

Backbone structure

A dominant consideration in the design of most antisense oligos has been to devise a structure that provides resistance to nucleases while still resembling natural nucleic acids as closely as possible. This conservative approach has spawned a number of DNA analogs (Fig. 1A) that may be unduly expensive for routine applications requiring systemic delivery. The high cost of DNA and its analogs is due in part to the low abundance of DNA in production-scale source material and the difficulty in cleaving DNA to the deoxyribonucleosides required for preparing DNA analogs. An additional factor in their high cost is the complexity and expense of coupling to hydroxyls, required in forming the phosphoester intersubunit linkages of most DNA analogs.

Rather than trying to solve inherent cost problems after a structural type has been developed, a better approach is to incorporate fundamental cost advantages in the initial structural design stage. Following this strategy, we reasoned that more affordable antisense oligos might be possible if inexpensive ribonucleosides could be exploited as starting material. The order-of-magnitude cost advantage of ribonucleosides relative to deoxyribonucleosides (Summerton, 1992) derives from the six-fold greater abundance of RNA relative to DNA in production-scale source material (e.g., yeast cake) and the ease of cleaving

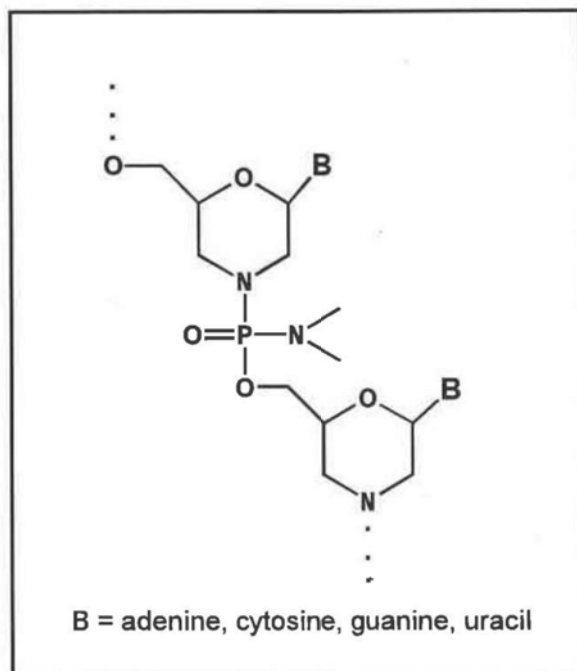


FIG. 2. Morpholino oligo structure.

RNA to its component ribonucleosides. It is noteworthy that ribonucleosides are now directly available from special excreting strains of yeast, further reducing their cost. However, the use of ribonucleosides for preparation of RNA and RNA analogs presents two serious problems. First, during oligo assembly, one must selectively couple either the 2' or the 3' hydroxyl. This is typically achieved in a relatively expensive manner by selectively masking the 2' hydroxyl with a cleavable or noncleavable moiety. The second problem is that coupling to the 3' hydroxyl of the riboside is even more difficult and expensive than the corresponding coupling of deoxyribonucleosides.

We envisioned that these problems could be circumvented by converting the riboside moiety to a morpholine moiety (Stirchak et al., 1989; Summerton, 1990) (Fig. 3). Although oligomers assembled from such Morpholino subunits differ substantially from DNA, RNA, and analogs thereof, our initial modeling studies carried out in 1985 suggested that such novel Morpholino-based oligomers might constitute useful and highly cost-effective antisense agents. The simple and inexpensive ribose to morpholine conversion shown in Figure 3 replaces two poor nucleophiles (the 2' and 3' hydroxyls) with a single good nucleophile (the morpholine nitrogen) and allows oligo assembly via simple and efficient coupling to the morpholine nitrogen without the expensive catalysts and postcoupling oxidation steps required in the production of most DNA-like antisense oligos. It is noteworthy that in spite of the relatively low nucleophilicity of the morpholine nitrogen ($pK_a = 5.75$), we still typically achieve coupling efficiencies of 99.7% without using catalysts.

Intersubunit linkage

We have assessed a substantial number of intersubunit linkage types, including the carbonyl, sulfonyl, and phosphoryl linkages (Fig. 4) (Summerton and Weller, 1991, 1993a,b; Stirchak et al., 1989). Although Morpholino oligos containing a number of such linkages provide effective binding to targeted genetic sequences, consideration of cost and ease of synthesis, chemical stability, aqueous solubility, and affinity and homogeneity of binding to RNA led us to focus on the phosphorodiamidate shown in Figure 2 as our principle linkage type for oligos targeted against single-stranded RNA sequences. These non-ionic phosphorodiamidate-linked Morpholino oligos exhibit quite good binding to complementary nucleic acids, particularly RNA sequences. Table 1 compares the temperature of melting (T_m) values at physiologic salt concentration for identical-sequence 20-mer oligos of three different antisense structural types paired with their complementary RNA. As seen in Table 1, RNA binding affinity is lowest for the phosphoroth-

ioate-linked DNA (S-DNA), appreciably higher for DNA, and highest for the Morpholino oligo.

PREPARATION

Oligo assembly

Although phosphorodiamidate-linked Morpholino oligos can be assembled by a variety of methods, one relatively simple method that has proved effective (Summerton and Weller, 1993b) entails protection and activation of the Morpholino subunit (Fig. 5A). The activated subunits can be stored at low temperatures for many months without significant breakdown. Whereas they are relatively resistant to hydrolysis, they react rapidly ($T_{1/2}$ of 1–2 minutes) with the morpholine nitrogen of growing chains on a 1% crosslinked polystyrene synthesis support loaded at 500 $\mu\text{M/g}$ of resin, with coupling efficiencies typically about 99.7%. A preferred oligo assembly cycle (Summerton and Weller, 1993b) is shown in Figure 5B. It is noteworthy that in large-scale syntheses, excess activated subunit used in the coupling step can be recovered and reused, effecting a further substantial reduction in production costs.

Because of cheaper starting materials and simpler, more efficient oligo assembly, we estimate that in large-scale production, the cost of these Morpholino antisense oligos will be at least an order of magnitude lower than the cost of corresponding DNA analogs (Summerton, 1992).

PROPERTIES

Solubility

For an antisense oligo to have effective access to its target sequence within the cytoplasm of a cell, the oligo should show reasonable water solubility. Good water solubility may also prove essential for systemic delivery of antisense oligos. Conventional wisdom in the antisense field is that non-ionic antisense oligos invariably show poor water solubility. In this regard, it is interesting that a Morpholino dimer containing a rigid carbamate linkage shows little or no base stacking (Kang et al., 1992), and in the absence of special solubilizing groups, Morpholino oligomers containing such carbamate linkages are quite insoluble in aqueous solutions (Stirchak et al., 1989). In contrast, phosphorodiamidate-linked Morpholino oligos of the type shown in Figure 2 show excellent base stacking (Kang et al., 1992) and are several orders of magnitude more soluble in aqueous solutions. To illustrate the exceptional aqueous solu-

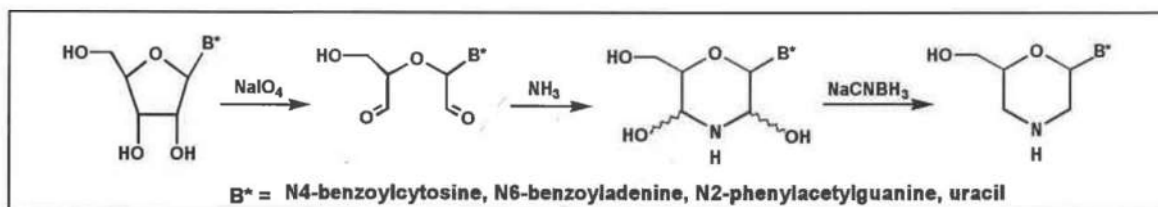


FIG. 3. Conversion of ribonucleoside to Morpholino subunit.

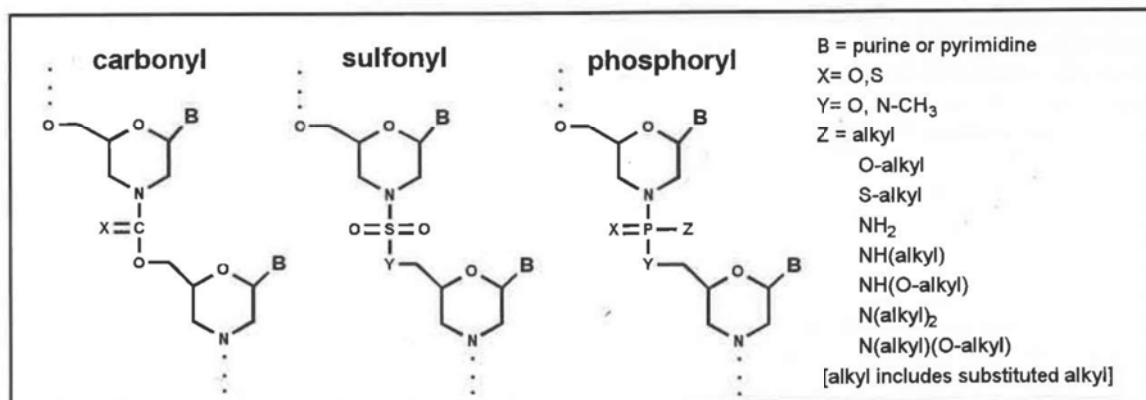


FIG. 4. Intersubunit linkage types for Morpholino oligos.

bility of Morpholino oligos of this type, we have dissolved 263 mg of a heteromeric 22-mer of the sequence 5'-GCUCGA-GACUUGUCCAUCAU in 1 ml of water (36 millimolar) at 20°C without reaching saturation.

We suggest that the poor water solubility of the carbamate-linked Morpholino oligos results at least in part from the difficulty of inserting the hydrophobic faces of the unstacked bases into an aqueous environment. In contrast, it seems likely that the excellent water solubility of the phosphorodiamidate-linked Morpholino oligos is a consequence of effective shielding of these hydrophobic faces from the polar solvent because of good stacking of the bases.

Biologic stability

To achieve reasonable efficacy, an antisense oligo should not be degraded rapidly either extracellularly or within cells. In this regard, it has been demonstrated that DNA and 2'-O-methyl RNA are rapidly degraded and phosphorothioate DNA is slowly degraded by nucleases in blood and within cells (Hoke et al., 1991; Morvan et al., 1993). Although resistance to nucleolytic degradation can be improved by adding special groups to the termini (Cazenave et al., 1987) or by incorporating a few nuclease-resistant intersubunit linkages near each end (Larrouy et al., 1992), we believe a better solution, on the basis of both function and cost, is to use a backbone structure that is inherently immune to a broad range of degradative enzymes present in the blood and within cells. A further advantage of using a backbone structure that is not degraded in the body is that it avoids concerns that modified nucleosides or nucleotides resulting from degradation of an antisense oligo might be toxic or might be incorporated into cellular genetic material and thereby lead to mutations or other undesired biologic effects.

In experiments detailed elsewhere (Hudziak et al., 1996), it is demonstrated that Morpholino phosphorodiamidate oligos of

the type shown in Figure 2 are immune to a wide range of nucleases, including DNase I (an endonuclease that cleaves both single-stranded and double-stranded DNA), DNase II (cleaves between the 5' oxygen and the phosphorus of DNA linkages), RNase A (cleaves on the 3' side of pyrimidines), RNase T1 (cleaves on the 3' side of guanines), nuclease P1 (cleaves single-stranded RNA and DNA), phosphodiesterase (3' exonuclease for both RNA and DNA), Mung bean nuclease (cleaves single-stranded RNA and DNA), and benzonase (cleaves both single-stranded and double-stranded RNA and DNA, including linear, circular, and supercoiled). These Morpholino oligos have also been found to be immune to pronase E, proteinase K, and pig liver esterase, as well as degradative enzymes in serum and a liver homogenate.

Antisense efficacy

Because of the excellent RNA binding affinity of oligos of this phosphorodiamidate-linked Morpholino structural type, it seemed likely Morpholino oligos would be effective in blocking translation of their targeted mRNAs, and this has been found to be the case. In cell-free translation experiments using a sensitive luciferase reporter, we have demonstrated that a Morpholino oligo 25 subunits in length, in both the presence and absence of RNase H, inhibits its targeted mRNA somewhat better than the corresponding S-DNA oligo in the presence of added RNase H, with both showing good efficacy at concentrations of 10 nM and above. Representative translational inhibition results are shown in Figure 6 (Summerton et al., 1997). A similar comparison of Morpholino and S-DNA antisense oligos targeted against murine tumor necrosis factor- α (TNF- α) mRNA in a cell-free translation system also showed greater activity for the Morpholino oligos (Taylor et al., 1996).

Specificity

In the early days of antisense research, one of the most compelling arguments for antisense therapeutics was their promise of exquisite specificity for their targeted genetic sequences. However, as the most synthetically accessible antisense structural types (DNA and S-DNA) have come into broad use, it has become clear that these two structural types provide reasonable

TABLE 1. MELTING TEMPERATURES OF RNA/OLIGO DUPLEXES

RNA/S-DNA	68.5°C
RNA/DNA	77.3°C
RNA/Morpholino	81.3°C

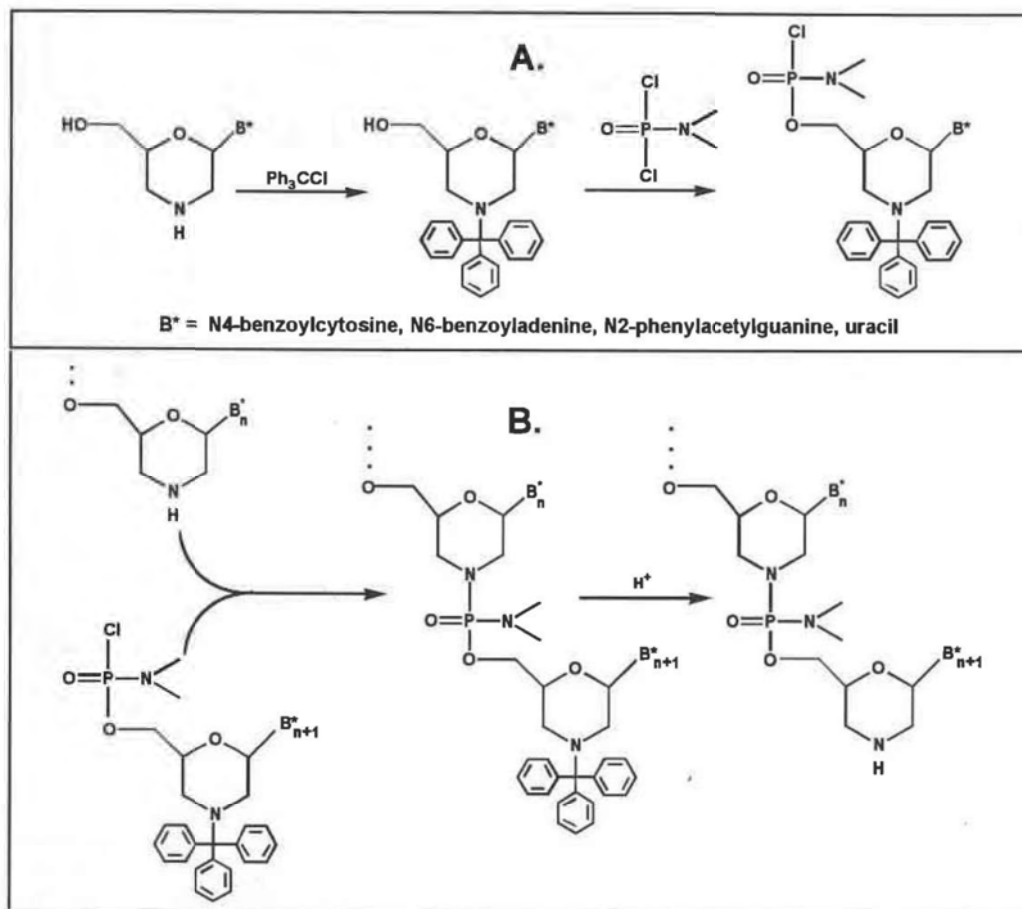


FIG. 5. Protection, activation, and coupling of Morpholino subunits.

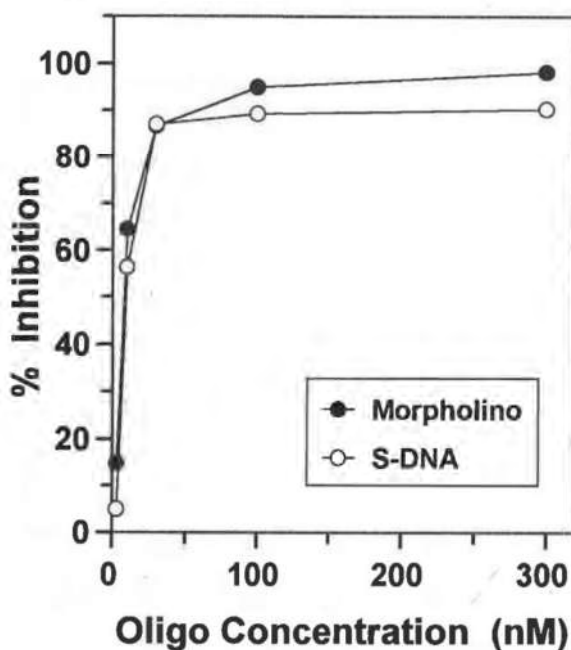


FIG. 6. Cell-free efficacy of Morpholino and S-DNA antisense oligos.

sequence specificity within only a very narrow concentration range (ANTIVIRALS Inc., 1993; Stein and Cheng, 1993).

We believe a key factor responsible for the low specificity of DNA and S-DNA oligos is their RNase H competency; that is, DNA and S-DNA form duplexes with complementary RNA that are readily cleaved by RNase H, an enzyme widely distributed in living organisms. The specificity problem arises because DNA/RNA and S-DNA/RNA duplexes as short as 5 base pairs in length are cleaved by RNase H (Crouch and Dirksen, 1982). Presuming about 6% of the genome is transcribed in higher animals, the patient's RNA pool will comprise about 200 million bases of unique-sequence RNA. With this level of sequence complexity, it is inevitable that antisense oligos will form many short transient duplexes with partially complementary nontarget sequences of inherent cellular RNAs. Cleavage of the RNA strand of such nontarget duplexes by endogenous RNase H (Larrouy et al., 1992; Cazenave et al., 1989) is expected to cause significant disruption of normal cellular translation. As this cleavage process releases the DNA or S-DNA in its original form, such oligos can continue the cycle of transiently pairing with additional nontarget cellular RNA sequences, cleavage of the RNA strand, and release of the antisense oligo. As a consequence, essentially every RNase H-competent oligo is expected to cleave hundreds to thousands of species of inherent cellular RNAs.

A second factor expected to contribute to superior specificity of Morpholino oligos relative to RNase H-competent types is that RNase H-independent oligos have far fewer potential targets in the inherent pool of cellular RNA. This is because most antisense structural types that do not support RNase H cleavage of their RNA targets have been found to be effective in blocking translation of their targeted mRNAs only when said oligos are complementary to sequences in the 5' leader region of that mRNA or when they are targeted against other special sites, such as splice junctions and transport signals [e.g., methylphosphonate DNA (Walder and Walder, 1988), α -DNA (Rayner et al., 1989), 2'-O-methyl RNA (Shibahara et al., 1989), and Morpholino (Summerton et al., 1997)]. We estimate that such special targetable regions constitute on the order of 2%–5% of the sequences in the cellular RNA pool. Presumably, this targeting limitation reflects the ability of ribosomes to displace essentially all antisense oligos during translocation down the coding region of mRNAs.

Because an antisense oligo that does not support RNase H cleavage cannot effectively block functioning of an RNA when said oligo is bound to sequences outside of special targetable regions, such an oligo only needs to distinguish its target sequence from those 2%–5% of the cellular RNA sequences comprising special targetable regions. In contrast, antisense oligos that form RNase H-cleavable duplexes with RNA can be effective when targeted essentially anywhere along an RNA transcript (Walder and Walder, 1988), presumably because RNase H cleavage at the target site of the antisense oligomer destroys the RNA, rendering moot the oligo displacement capability of translocating ribosomes. Accordingly, RNase H-competent oligos (DNA and S-DNA) face the much greater specificity challenge of distinguishing selected target sequences from essentially the entire pool of cellular RNA sequences. As a consequence, RNase H-independent oligos, such as Morpholinos, should enjoy a 20-fold to 50-fold advantage in sequence

specificity because of this more than order-of-magnitude reduction in the number of inherent nontarget cellular sequences of any given length that they can inhibit.

A third factor compromising the specificity of S-DNA oligos is their promiscuous binding to proteins (Krieg and Stein, 1995), including components of the cell's replication, transcription, and translation machinery.

Given these factors expected to limit the sequence specificity of RNase H-competent antisense structural types, particularly S-DNA, we set out to compare sequence specificities of S-DNA and Morpholino antisense oligos. To this end, we carried out stringent specificity assays in a cell-free translation system using two oligos of each structural type (Summerton et al., 1997). In these experiments, one oligo was perfectly complementary to its target mRNA to provide a measure of the total inhibition afforded by that oligo type. The other oligo incorporated 4 mispairs to that same mRNA target sequence, with the longest run of perfect pairing comprising 10 contiguous base pairs, to provide an estimate of the low-specificity component of the inhibition. The difference between these two inhibition values at each concentration then provided a measure of the high-specificity component, which we denote as "sequence-specific inhibition."

Figure 7 (experimental as in Summerton et al., 1997) shows that the S-DNA oligo achieved reasonable efficacy at concentrations above about 10 nM, but the sequence-specific component of its inhibition dropped below 50% at concentrations of only 100 nM and higher. The corresponding Morpholino oligo achieved even better efficacy at 10 nM while maintaining good sequence specificity through 10,000 nM, the highest concentration tested. Thus, in this stringent test of specificity, the Morpholino oligo achieved highly effective and specific antisense activity over a concentration range more than two orders of magnitude greater than the concentration range wherein the corresponding S-DNA achieved reasonable efficacy and specificity.

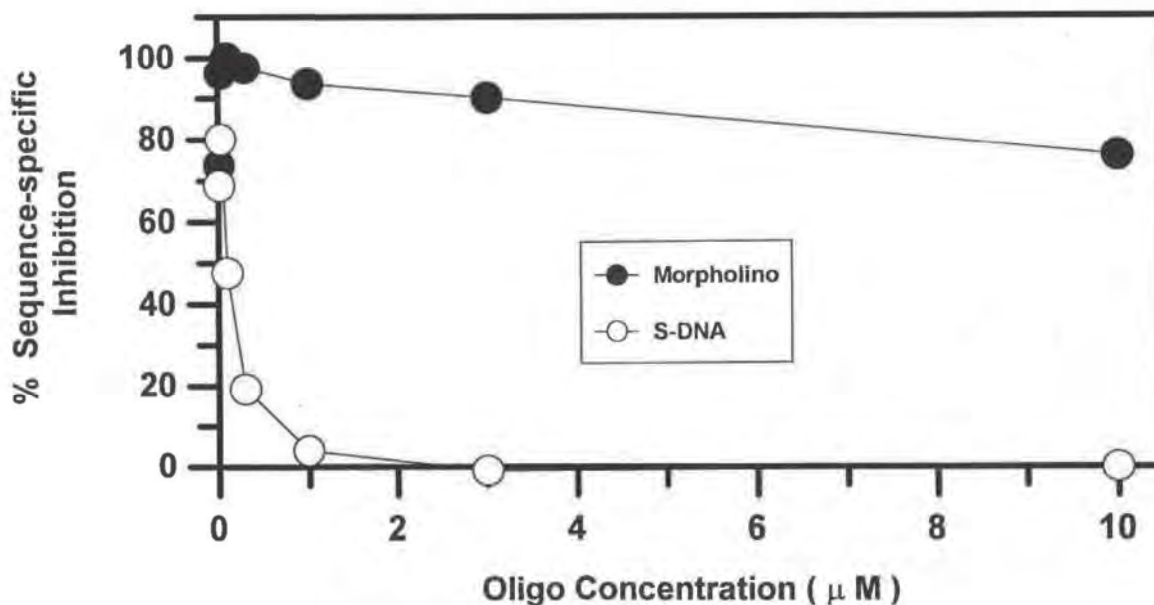


FIG. 7. Sequence specificity of Morpholino and S-DNA oligos.

Taylor et al., (1996) have reported that S-DNAs targeted against TNF- α mRNA showed very poor sequence specificity in a cell-free translation system, whereas the corresponding Morpholino oligos afforded good specificity over the full range tested.

Activity in cells

For effective biologic activity, an antisense oligo must gain entry into the cellular compartments where the target genetic sequence is synthesized, processed, and functions—specifically, the cytosol/nuclear compartment. Our experiments with fluorescent-tagged Morpholino oligos suggested that these oligos enter mammalian cells by what appears to be endocytosis, but they do not appear to subsequently cross the endosomal or lysosomal membrane into the cytosol, based both on visualization of fluorescent-tagged oligos and a functional assay employing a transfected plasmid (Partridge et al., 1996). This result is in agreement with limitations on uptake of antisense oligos reported by others. Specifically, a number of studies have been reported that suggest that in the absence of experimental manipulations that compromise the cell membrane, both polyanionic oligos [e.g., S-DNA (Wagner et al., 1993; Tonkinson and Stein, 1994) and 2'-O-methyl RNA (Oberhauser and Wagner, 1992)] and non-ionic oligos [e.g., methylphosphonate DNA (Shoji et al., 1991) and PNAs (Bonham et al., 1995)] enter cells primarily or exclusively by endocytosis. Further, a number of studies on a variety of antisense structural types indicate that most or all of the antisense oligo that gains entry by endocytosis does not subsequently traverse

the endosomal or lysosomal membrane to enter in an intact form into the cytosol, where protein synthesis occurs (Oberhauser and Wagner, 1992; Shoji et al., 1991; Bonham et al., 1995).

However, we have found that antisense oligos can be easily delivered into cultured cells simply by passaging anchorage-dependent cells by the common procedure of scraping with a rubber policeman. This has been shown to achieve significant oligo entry into the cytosolic compartment if the oligo is present during the scraping (Partridge et al., 1996). Further, Morpholino oligos delivered into cells by such scrape loading show good activity and specificity therein, whereas corresponding S-DNA oligos (both antisense and control sequences) largely fail to inhibit their targets within scrape-loaded cells at concentrations up to 3 μ M in the medium and instead are often stimulatory (Summerton et al., 1997). Figure 8 shows a comparison of the activities of representative Morpholino and S-DNA oligos in scrape-loaded cells (experimental as in Summerton et al., 1997).

Taylor et al. (1996) have also compared the activity of S-DNA and Morpholino antisense oligos in cultured cells. In their studies, the S-DNAs were delivered into mouse macrophage-like cells (RAW 264.7) using lipofectin. Both oligo types were targeted against TNF- α mRNA, and treated cells were assessed for inhibition of lipopolysaccharide-induced TNF- α production. In agreement with our in-cell results, Taylor et al. report that both the antisense and control S-DNAs stimulated instead of inhibited TNF- α production, whereas the Morpholino antisense oligo, although poorly delivered into the cells, afforded significant and specific inhibition of TNF- α production.

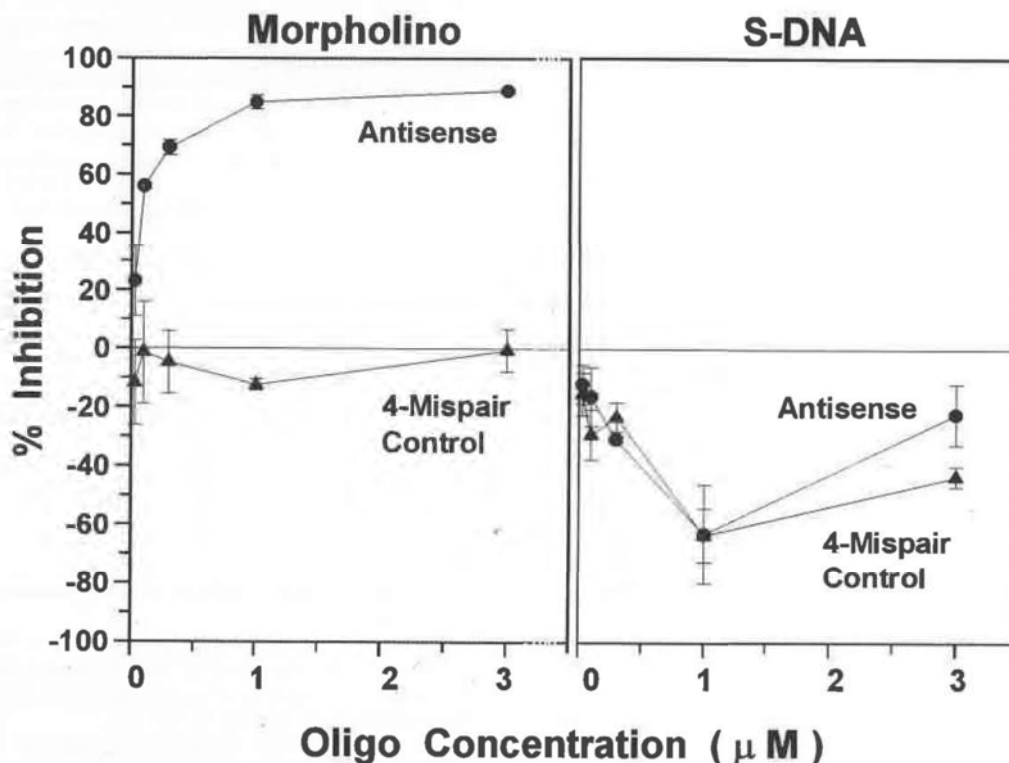


FIG. 8. In-cell activities of Morpholino and S-DNA oligos.

In vivo properties

To date, our principal efforts have focused on optimizing the Morpholino structural type and on studying the properties of Morpholino oligos at the biophysical level, in cell-free translation systems, and in cultured cells. In light of the promising results from those studies, we and several collaborators are now shifting our focus to *in vivo* studies.

A very preliminary ranging study was carried out to assess acute toxicity. In this study, a representative 20-mer Morpholino oligo in phosphate-buffered saline was injected intravenously into mice at doses ranging from 88 mg/kg to 700 mg/kg. No acute toxicity was seen at any of these doses. However, over a period of 2 weeks, an effect on body weight gain and ruffled coat was observed at the highest dose. Using the results from this ranging study, an extensive toxicity study has been initiated and will be the subject of a future report.

In addition, a variety of efficacy studies in mice and rats are in progress to assess the possible use of Morpholino oligos for therapeutic applications. We are also investigating possible methods for improving the delivery of these oligos into the cytosol/nuclear compartment of cells *in vivo*.

DISCUSSION

Morpholino oligos meet key requirements for safe, effective, and affordable antisense therapeutics, including high efficacy at low nanomolar concentrations, high sequence specificity over a thousandfold concentration range, little or no nonantisense activity, total stability in blood and within cells, excellent water solubility, and low production costs relative to other antisense structural types. Our efforts are now focused on achieving effective delivery into the cytosol/nuclear compartment of cells by means suitable for therapeutic applications and on studying the activities of these oligos in animals.

ACKNOWLEDGMENTS

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Exhibit 3

1 IN THE UNITED STATES DISTRICT COURT

2 FOR THE DISTRICT OF DELAWARE

3 - - - - - x
NIPPON SHINYAKU CO., LTD., :
4 : C.A. No. 21-1015
Plaintiff, : (CBW)
5 :
vs. :
6 :
SAREPTA THERAPEUTICS, INC., :
7 :
Defendant. :
8 - - - - - x
SAREPTA THERAPEUTICS, INC., :
9 :
and THE UNIVERSITY OF WESTERN :
10 :
AUSTRALIA, :
11 :
Defendants/Counter-Plaintiffs, :
12 :
vs. :
13 :
NIPPON SHINYAKU CO., LTD. :
14 :
and NS PHARMA, INC., :
15 :
Plaintiffs/Counter-Defendants.:
16 _____ x

17 
18 Videotaped Deposition of MICHELLE HASTINGS, Ph.D.

19 Chicago, Illinois

20 Friday, September 13, 2024

21 9:05 a.m.

22
23 Job No.: 553177

24 Pages: 1 - 134

25 Reported By: JENNIFER L. BERNIER, CSR, RMR, CRR

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

13

1	Dr. Wood?	09:14:38
2	A I would -- no. I would say that we have	09:14:39
3	similar expertise. He has worked more directly with	09:14:42
4	Duchenne's muscular dystrophy and -- or antisense	09:14:46
5	oligonucleotides directed towards skipping of the	09:14:51
6	exons in -- in dystrophin and published on those.	09:14:54
7	Q And so -- so you'd defer to his opinions	09:15:00
8	when it comes to treating Duchenne's muscular	09:15:02
9	dystrophy?	09:15:06
10	A I don't think I have opinions about treating	09:15:07
11	patients with Duchenne's muscular dystrophy. So he	09:15:10
12	does, as far as he talks about that.	09:15:15
13	Q And did you speak with Dr. Wood in	09:15:19
14	connection with preparing your reports, the 101 and	09:15:23
15	102 reports?	09:15:27
16	A I did not speak with Dr. Wood directly.	09:15:28
17	Q Any particular reason why not?	09:15:32
18	A Well, we had --	09:15:39
19	MS. LO: I'm going to actually interpose an	09:15:39
20	objection. I'll caution Dr. Hastings not to divulge	09:15:43
21	the substance of any attorney-client communications	09:15:46
22	in her response.	09:15:50
23	You can answer the question to the extent	09:15:51
24	you can do that.	09:15:53
25	MR. FRAZIER: Right, and I'll rephrase the	09:15:54

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

14

1	question.	09:15:55
2	BY MR. FRAZIER:	09:15:56
3	Q To be clear, I'm not asking about the	09:15:56
4	attorneys. This is your analysis that's reflected	09:15:58
5	in Exhibits 101 and 102, right?	09:16:01
6	A Right.	09:16:04
7	Q And in conducting your analysis, you relied	09:16:05
8	on Dr. Wood, correct?	09:16:07
9	A No. I mean, I --	09:16:08
10	Q That's not correct?	09:16:11
11	A I mean, I refer to some of his -- his	09:16:12
12	opinions in mine and agree with his opinions in my	09:16:14
13	reports.	09:16:22
14	Q But you didn't rely on any of those	09:16:22
15	opinions?	09:16:24
16	A I agreed with his opinions.	09:16:25
17	Q Did you rely on any of the opinions of	09:16:26
18	Dr. Wood in your report?	09:16:28
19	A Can -- which opinions?	09:16:29
20	Q I'm just asking -- you prepared the report.	09:16:30
21	Did you rely on all your own opinions or also the	09:16:32
22	opinions of Dr. Wood?	09:16:35
23	A I just -- can you -- I would like to know	09:16:36
24	what opinions. I have -- I might have. I just	09:16:38
25	can't remember all of the opinions that I -- when I	09:16:41

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

15

1	referred to Dr. Wood.	09:16:45
2	Q So you don't know, as you sit here today	09:16:46
3	with your reports, whether you relied on any	09:16:49
4	opinions of Dr. Wood or not?	09:16:52
5	A Well, should I look at them?	09:16:53
6	Q You -- you can, but I -- it's really not to	09:16:55
7	look up. It's -- you did the analysis. The	09:16:58
8	question is: In doing your analysis, do you recall	09:17:00
9	relying on the opinions of Dr. Wood?	09:17:03
10	A I -- I recall agreeing with his opinions.	09:17:06
11	Q But not relying on them?	09:17:10
12	A In most cases, we have similar expertise in	09:17:11
13	terms of use of the antisense oligonucleotides. And	09:17:15
14	he agreed with me and I agreed with him on our -- on	09:17:18
15	our opinions.	09:17:22
16	Q All right. But you didn't talk to him?	09:17:22
17	A I mean, I read his reports and -- and	09:17:25
18	through the process of his -- of his different	09:17:31
19	versions. We did not talk directly.	09:17:35
20	Q You read different versions of his report?	09:17:39
21	A As they were being -- as they were being	09:17:43
22	edited.	09:17:46
23	Q About how many different versions of	09:17:47
24	Dr. Wood's report do you recall reviewing?	09:17:49
25	A I don't remember. I don't remember.	09:17:51

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

16

1	Q More than one?	09:17:53
2	A I -- there was a version before the final	09:17:54
3	version at least, yeah.	09:17:57
4	Q At least one, maybe more than one --	09:18:00
5	A I don't recall.	09:18:03
6	Q -- version before the final version?	09:18:04
7	A I don't recall. I believe we typically have	09:18:05
8	a -- have a -- have a draft that goes out; and then	09:18:08
9	there is a final draft that I get as a -- you know,	09:18:11
10	as the final one that we -- that we have here. And	09:18:14
11	I know -- I couldn't say how much it changed between	09:18:19
12	them. I didn't compare.	09:18:21
13	Q But it's certainly something you considered	09:18:23
14	in preparing your report?	09:18:26
15	A I had it available to me when I prepared my	09:18:27
16	report. I made the first draft of my report before	09:18:33
17	I had his available. I can't remember the exact	09:18:39
18	sequence.	09:18:43
19	MR. FRAZIER: All right. And, counsel, I'd	09:18:44
20	ask for you to produce the -- that NS produce any	09:18:45
21	copies -- draft copies of Dr. Wood's report that	09:18:50
22	were reviewed and considered by Dr. Hastings in	09:18:53
23	connection with preparation of her reports.	09:18:57
24	MS. LO: We'll take that request under	09:18:58
25	consideration if you make it in writing after the	09:19:00

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1	(Reporter clarification.)	09:53:16
2	MS. LO: Objection to form.	09:43:07
3	THE WITNESS: For -- in this case -- well,	09:43:08
4	in particular for the numbers that I was	09:43:12
5	calculating, it was within what the genus was that	09:43:13
6	we were -- that I was referring to, which was a 10	09:43:18
7	to the billion number that we had talked about. So	09:43:20
8	that's within that -- within the claimed species.	09:43:25
9	BY MR. FRAZIER:	09:43:29
10	Q But you're -- you're commenting, among other	09:43:29
11	things, on whether there is sufficient targeting of	09:43:31
12	a particular area of exon 53, correct?	09:43:35
13	A Are we talking now about the whole exon?	09:43:42
14	Q Part of your opinion is based on the idea	09:43:48
15	that the region that Dr. Wilton recites in	09:43:51
16	claim 1 --	09:43:59
17	A Yes.	09:43:59
18	Q -- is a very large region, if I'm	09:43:59
19	understanding your opinions correctly, correct?	09:44:02
20	MS. LO: Objection.	09:44:05
21	THE WITNESS: The -- I would say the region	09:44:06
22	isn't large for the claim. The sequence is a	09:44:07
23	limited sequence, but the possibilities of how you	09:44:11
24	can target that sequence, the modifications that you	09:44:16
25	can do on the oligonucleotides which is akin to how	09:44:18

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1	you can modify the wings of an -- of a flying	09:44:22
2	structure is similar.	09:44:25
3	BY MR. FRAZIER:	09:44:41
4	Q The size of exon 53 is approximately 212	09:44:42
5	bases; is that right?	09:44:45
6	A Well, did you find it somewhere?	09:44:46
7	Q I'm just asking you.	09:44:47
8	A Okay. It could take a long time looking for	09:44:49
9	this because I am sure it is in here somewhere.	09:44:54
10	But to be clear again, like the analogy is	09:44:58
11	referring to -- in addition to all the modifications	09:45:00
12	that can be done, you establish there is wings.	09:45:03
13	This is a region; and then everything you can do to	09:45:08
14	those wings to see if it will fly, if it has	09:45:11
15	activity, right?	09:45:15
16	He didn't show that he could -- that it	09:45:15
17	could fly at all, right? I mean, there is no	09:45:18
18	activity in any of the evidence that I saw at the	09:45:20
19	time that he filed the patent. But he then claimed	09:45:23
20	a whole bunch of modifications.	09:45:27
21	Q So -- well, let's -- let's pursue a little	09:45:29
22	bit of that. I can't resist.	09:45:34
23	So you said Dr. Wilton -- there was no	09:45:37
24	activity at all in any of the evidence that you saw	09:45:43
25	at the time he filed the patent; is that correct?	09:45:47

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1	A Yeah. I mean, I think that he had listed --	09:45:52
2	there was no primary data in the patent showing that	09:45:58
3	there was activity of that. There was faint	09:46:00
4	skipping in the one -- the one pseudospecies that	09:46:04
5	they claimed. So he had not shown that the claim --	09:46:11
6	any -- any oligonucleotide of the claim had any	09:46:13
7	activity in that -- in 2005.	09:46:18
8	Q Right. And so -- so is it -- is it your	09:46:19
9	view that in addition to describing the invention of	09:46:22
10	claim 1, that Dr. Wilton needed to prove to those of	09:46:29
11	skill in the art that it would work?	09:46:34
12	A Well, that's part of the claim, that it	09:46:38
13	would induce exon 53 skipping.	09:46:40
14	Q Right. So is -- is the answer to my	09:46:42
15	question yes?	09:46:46
16	MS. LO: Objection. Mischaracterizes.	09:46:47
17	THE WITNESS: Well, the claim says that	09:46:52
18	you'd have this --	
19	(Reporter clarification.)	09:46:59
20	THE WITNESS: Okay. All right.	09:46:59
21	The claim says that it would have -- you	09:46:59
22	know, describes the structure and then says that it	09:47:01
23	would induce exon 53 skipping as part of the claim.	09:47:03
24	BY MR. FRAZIER:	09:47:07
25	Q All right. And my question is: As part of	09:47:07

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1	your analysis, was your analysis that in addition to	09:47:09
2	describing the invention of claim 1, that Dr. Wilton	09:47:12
3	needed to prove to those of skill in the art that it	09:47:16
4	would work?	09:47:20
5	A I'd -- I'm afraid I don't understand that	09:47:21
6	question very well, actually. He's claiming that	09:47:26
7	he's making antisense oligonucleotides to induce	09:47:30
8	exon 53 skipping.	09:47:34
9	Q Right. So, for example, let's look at	09:47:35
10	column 65 of the '851 patent, which is marked as	09:47:38
11	Hastings Exhibit 103.	09:47:41
12	A Did you say page 65?	09:47:55
13	Q Column 65.	09:47:57
14	A Column 65. I got it.	09:47:59
15	Q And column 65 reports a number of antisense	09:48:13
16	oligonucleotides and their ability to induce	09:48:19
17	skipping, correct?	09:48:24
18	A Yes, that's right.	09:48:25
19	Q All right. And, for example, at Sequence	09:48:26
20	ID 195, there is an oligonucleotide that's labeled	09:48:34
21	+23 to +47, right?	09:48:41
22	A Yes.	09:48:42
23	Q And according to the patent, it reports very	09:48:42
24	faint skipping, correct?	09:48:46
25	A That's what it says, yes.	09:48:47

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1	Q All right. And you agree that a person of	09:48:48
2	skill in the art would understand what that	09:48:50
3	oligonucleotide is, correct?	09:48:52
4	A You mean the sequence of it?	09:48:54
5	Q Yes.	09:48:59
6	A It has -- the sequence of the	09:49:01
7	oligonucleotide is written there, yes.	09:49:03
8	Q Okay. And if a person of skill in the art	09:49:05
9	made an oligonucleotide corresponding to Sequence	09:49:13
10	ID 195, +23 to +47, and tested it in a standard	09:49:19
11	skipping assay, you would anticipate that they	09:49:25
12	would, in fact, see skipping, right?	09:49:31
13	MS. LO: Objection. Incomplete	09:49:32
14	hypothetical.	09:49:34
15	THE WITNESS: What I -- when -- back to my	09:49:34
16	original comment about not having evidence, this,	09:49:35
17	again, was done with a -- with not the chemistry	09:49:38
18	that was claimed. And also having the privilege of	09:49:43
19	looking at [REDACTED] and	09:49:47
20	what was published here, I don't see evidence that	09:49:50
21	this was skipping.	09:49:53
22	BY MR. FRAZIER:	09:49:53
23	Q That's not my question. My question was:	09:49:54
24	If a person of ordinary skill in the art followed	09:49:57
25	the patent and made an oligonucleotide corresponding	09:49:59

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1	to Sequence ID No. 1, +23 to +47, you would expect	09:50:03
2	that that would actually work for skipping, right?	09:50:09
3	MS. LO: Objection. Incomplete	09:50:11
4	hypothetical.	09:50:13
5	THE WITNESS: When I was evaluating this, I	09:50:13
6	would say that the evidence here -- at that time, I	09:50:17
7	don't think there was evidence that it would cause	09:50:22
8	skipping.	09:50:25
9	BY MR. FRAZIER:	09:50:25
10	Q Right. I'm going to need you to answer my	09:50:25
11	question. All right?	09:50:28
12	A Oh, can you repeat it?	09:50:28
13	Q My question was: If a person of ordinary	09:50:29
14	skill in the art were to make an oligonucleotide	09:50:31
15	corresponding to Sequence ID 195, you would expect	09:50:36
16	based on your own work in this case that they would	09:50:41
17	see skipping, right?	09:50:44
18	A Oh, I see what you're asking me.	09:50:45
19	When I made an antisense oligonucleotide	09:50:48
20	195, +23 to +47, for -- and tested it with the	09:50:52
21	modern reagents that we had, we did see skipping.	09:50:58
22	Q Right. And -- and to be clear, when you did	09:51:03
23	your testing, you were attempting to reproduce the	09:51:06
24	conditions set forth in the '851 patent, right?	09:51:11
25	A Yes. But I -- to be very clear, I designed	09:51:14

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1 that not 100 percent knowing whether or not it was 09:51:17
2 going to cause skipping, which is why I did the 09:51:20
3 experiment. I did not have -- see evidence that it 09:51:22
4 had -- that it was. So I -- you know, I wasn't 09:51:24
5 sure, actually. 09:51:25

6 Q Right. But you did see the patent said that 09:51:26
7 there was going to be skipping, right? That's what 09:51:29
8 the patent -- 09:51:31

9 A When I say the data -- 09:51:34

10 THE REPORTER: One at a time, please.

11 THE WITNESS: Right.

12 Yeah, and I also saw [REDACTED] 09:51:37
13 [REDACTED]. Because as a scientist, you know, 09:51:40
14 anybody can say very faint skipping. It becomes 09:51:43
15 a -- a little bit qualitative, right? Subjective. 09:51:45

16 So I saw this. I also saw [REDACTED] 09:51:50

17 [REDACTED] 09:51:55

18 [REDACTED] 09:51:58

19 [REDACTED] So to me, I -- I actually half expected 09:52:00
20 it not to skip in my work. 09:52:06

21 BY MR. FRAZIER:

22 Q Right. And so that's -- that takes us back 09:52:08
23 to my prior question, which then you concluded for 09:52:11
24 your written description analysis that not only does 09:52:16
25 the patent have to describe the invention, it also 09:52:21

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1 has to have enough data to convince someone of skill 09:52:27
2 that the invention would work? 09:52:32
3 MS. LO: Objection. Mischaracterizes. 09:52:34
4 THE WITNESS: I think it has to describe it 09:52:36
5 and it has to demonstrate that there is exon 53 09:52:38
6 skipping. I don't know about -- it has to -- it has 09:52:43
7 to skip. And this one, I did not see that there was 09:52:46
8 evidence. I mean, they can write what they want 09:52:52
9 there. 09:52:54
10 BY MR. FRAZIER: 09:52:54
11 Q And they were right, right? They were 09:52:55
12 correct, that one skips? 09:52:57
13 A But they didn't have -- 09:52:58
14 MS. LO: Objection. 09:52:58
15 THE WITNESS: [REDACTED] 09:52:59
16 [REDACTED]. So 09:53:03
17 they wrote this; but whether or not it was -- [REDACTED] 09:53:07
18 [REDACTED] 09:53:10
19 [REDACTED] -- and certainly not for the 09:53:12
20 PMO, which is what they claimed. 09:53:18
21 BY MR. FRAZIER: 09:53:20
22 Q Right. So you agree that also if a person 09:53:20
23 of skill in the art were to make a PMO version of 09:53:23
24 Sequence ID 195 having the characteristics of 09:53:29
25 claim 1 where the -- the user tees, that would also 09:53:33

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1	result in skipping, right?	09:53:39
2	MS. LO: Objection. Incomplete	09:53:42
3	hypothetical.	09:53:44
4	THE WITNESS: At the time of this claim,	09:53:44
5	[REDACTED]	09:53:45
6	[REDACTED].	09:53:46
7	BY MR. FRAZIER:	09:53:51
8	Q But you know, in fact, it -- it would skip;	09:53:53
9	that it has subsequently been demonstrated that a	09:53:54
10	PMO version of that oligonucleotide as set forth in	09:54:00
11	the patent does skip, correct?	09:54:03
12	MS. LO: Objection to form.	09:54:04
13	THE WITNESS: I mean, in 2020, 50 years --	09:54:05
14	15 years later, we made one and -- and had -- and it	09:54:11
15	did skip. In 2005, there was no evidence.	09:54:14
16	BY MR. FRAZIER:	09:54:17
17	Q I hear you on that, but -- strike that.	09:54:19
18	Likewise, if we go to Sequence ID No. 193,	09:54:23
19	it reports that an oligonucleotide from +39 to +69	09:54:30
20	results in strong skipping, right?	09:54:37
21	A Yes. Mm-hmm.	09:54:40
22	Q And you agree that if a person of ordinary	09:54:42
23	skill in the art made an oligonucleotide according	09:54:44
24	to claim 1 of the '851 patent corresponding to	09:54:47
25	Sequence ID 193, you would expect they would observe	09:54:51

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1	caused skipping?	10:00:09
2	BY MR. FRAZIER:	10:00:09
3	Q Yeah.	10:00:10
4	A I think that, yeah, other groups did test	10:00:11
5	that afterwards and showed skipping for the -- for	10:00:14
6	the PMO, I believe.	10:00:19
7	Q Right. But I'm not --	10:00:21
8	A But in 2005, I -- it wasn't clear when they	10:00:21
9	filed the patent.	10:00:25
10	Q My question to you is: A person of ordinary	10:00:26
11	skill in the art reads the '851 patent, including	10:00:31
12	the report that SEQ ID 195, +23 to +47, an	10:00:36
13	oligonucleotide shows skipping. And they go out and	10:00:45
14	they test it, and they see skipping. That's not a	10:00:47
15	discovery, right? That's confirmation?	10:00:51
16	A Well, I think --	10:00:52
17	MS. LO: Objection to form.	10:00:52
18	Sorry.	10:00:55
19	THE WITNESS: Yeah. I think what you have	10:00:55
20	to understand is at that time when you have Matsuo,	10:00:56
21	who had done screening of a number of	10:01:02
22	oligonucleotides around exon 53 and had a lot of	10:01:05
23	inactive ones and then you have this, which has	10:01:10
24	skipping oligos all across the exon, I don't know	10:01:14
25	that -- and that -- and furthermore, the -- the very	10:01:18

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1	faint skipping with a non-PMO oligonucleotide, I	10:01:21
2	think those things all together considered, I don't	10:01:27
3	know that it is evidence that anybody would have	10:01:31
4	expected that 23 to 47 would have -- would have	10:01:36
5	worked or, furthermore, that there was a hotspot in	10:01:39
6	that region.	10:01:41
7	BY MR. FRAZIER:	10:01:42
8	Q So you think that somebody coming along	10:01:43
9	and -- and after reading the patent, testing	10:01:45
10	Sequence ID 195, +23 to +47, and observing skipping,	10:01:51
11	that would be a discovery on their part and not	10:01:56
12	something they got from Dr. Wilton?	10:01:59
13	MS. LO: Objection to form.	10:02:03
14	THE WITNESS: I think that he just didn't	10:02:04
15	have evidence that it caused skipping there. He	10:02:07
16	said he did, but it was really not. [REDACTED]	10:02:11
17	[REDACTED]	10:02:14
18	[REDACTED]	10:02:19
19	[REDACTED]	10:02:24
20	BY MR. FRAZIER:	10:02:27
21	Q I believe we started out here by saying, you	10:02:27
22	know, the -- the DMD gene is very large, millions of	10:02:30
23	bases, right?	10:02:35
24	A Mm-hmm.	10:02:36
25	Q And that this particular oligo +23 to +47 is	10:02:37

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1	this tiny, tiny fraction; and yet, it causes	10:02:46
2	skipping, right?	10:02:51
3	A Are we talking just about the exon 53?	10:02:51
4	Q We're talking about +23 to +47 as compared	10:02:54
5	to exon 53 or the entire gene. It's a very specific	10:02:59
6	oligonucleotide, right?	10:03:06
7	A I do -- I just -- yeah, I -- it definitely	10:03:07
8	is very specific. I'm just not sure what the	10:03:11
9	relevance to the whole entire gene is. Because, I	10:03:14
10	mean, in the antisense oligonucleotide field, you	10:03:18
11	have to a -- I mean, it's a very complex process,	10:03:20
12	right, of exon skipping.	10:03:24
13	It goes beyond just base pairing. You have	10:03:25
14	to consider the splicing reaction, the structure of	10:03:28
15	the RNA. So I'm not quite sure what the question is	10:03:32
16	that you're asking.	10:03:35
17	Q Well, I'm -- I'm asking about what	10:03:36
18	Dr. Wilton and his studies in the '851 patent	10:03:38
19	contributed to the field. And with respect to the	10:03:41
20	field, he contributed that exon 53, this particular	10:03:45
21	oligonucleotide +23 to +47, showed skipping. That's	10:03:51
22	something he told the field in the '851 patent,	10:03:56
23	right?	10:03:59
24	A He wrote -- I mean, he wrote that. I mean,	10:03:59
25	I think he showed strong skipping for 193. Again,	10:04:02

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1	yes, he wrote that here, that there was faint	10:04:10
2	skipping.	10:04:13
3	Q Right.	10:04:13
4	A My opinion is that there was no evidence	10:04:13
5	that there was faint skipping.	10:04:15
6	Q Right. But if a person of ordinary skill in	10:04:17
7	the art had followed your opinion and not followed	10:04:20
8	up on that, they would have been wrong, correct?	10:04:23
9	MS. LO: Objection. Vague.	10:04:27
10	THE WITNESS: I -- I don't think so.	10:04:33
11	Because 195 wasn't -- didn't end up being a -- you	10:04:35
12	know, what -- didn't end up being a -- a drug,	10:04:42
13	right? I mean, people went afterwards and screened	10:04:44
14	the entire exon with many, many oligonucleotides and	10:04:48
15	different chemistries. So they didn't really learn	10:04:52
16	the evidence after this.	10:04:54
17	It didn't seem to me that they were really	10:04:55
18	learning anything from this; rather, they were maybe	10:04:58
19	taking guidance from the fact that all across this	10:05:01
20	exon, there were some things that worked and some	10:05:03
21	things that didn't, right? And -- and that was also	10:05:05
22	true from Matsuo, from -- from Aartsma-Rus	10:05:10
23	beforehand. There was just unpredictability. And	10:05:15
24	this just heightens that unpredictability, in my	10:05:19
25	opinion.	10:05:25

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1	BY MR. FRAZIER:	10:05:28
2	Q You're aware that NS, when it began its	10:05:28
3	studies, looked to the Wilton patent?	10:05:32
4	MS. LO: Objection. Mischaracterizes.	10:05:34
5	THE WITNESS: I -- I don't know.	10:05:36
6	BY MR. FRAZIER:	10:05:41
7	Q You've reviewed internal NS documents	10:05:41
8	relating to their work?	10:05:45
9	A Yes.	10:05:46
10	Q Okay. And so you -- you understand that at	10:05:46
11	the start of their project, they were looking to the	10:05:48
12	to the -- to the Wilton patent?	10:05:53
13	MS. LO: Objection.	10:05:55
14	THE WITNESS: I might need to see those	10:05:57
15	documents just to refresh my memory.	10:05:59
16	BY MR. FRAZIER:	10:06:47
17	Q Let me show you what's been previously	10:06:48
18	marked as Exhibit Watanabe 15A.	10:06:50
19	(Exhibit 15A, previously marked, is	
20	attached to the transcript.)	10:07:01
21	MS. LO: I'm going to object as outside the	10:07:01
22	scope of the supplemental expert reports.	10:07:03
23	MR. FRAZIER: I disagree. I think it goes	10:07:11
24	to -- precisely to the opinions that she's	10:07:14
25	providing, that a person of ordinary skill in the	10:07:16

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1	'851 patent, and look at claim 1 of that.	10:32:23
2	A Okay.	10:32:37
3	Q All right. And so first of all, you're	10:32:41
4	indicating that the claimed ASOs may have various	10:32:43
5	chemical structures. And if I understand correctly,	10:32:48
6	one of the structures that you say they have -- they	10:32:51
7	can have is modified bases; is that correct?	10:32:53
8	A Yes.	10:32:56
9	Q All right. And -- and so if you just point	10:32:57
10	me in claim 1, where in claim 1 does it say that you	10:32:59
11	can have modified bases?	10:33:05
12	A Oh, yeah. And just relating to my last --	10:33:07
13	I'm -- I'm not saying that obviously it cannot	10:33:11
14	modify the base. I'm just referring to the	10:33:15
15	specification saying that it can have modified	10:33:17
16	bases. So I just wanted to amend my previous	10:33:21
17	answer.	10:33:24
18	But so for this one, you're asking about	10:33:25
19	where it says in the claim?	10:33:28
20	Q Right.	10:33:31
21	A In the claim 1, it doesn't talk about	10:33:32
22	modified bases in the specification. It talks	10:33:35
23	about -- it has a long list of all the modifications	10:33:38
24	that can be added to antisense oligonucleotides.	10:33:40
25	Q Right. And -- and how would you	10:33:44

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1	characterize those -- those various modifications,	10:33:46
2	or what have you, that are in the -- in the -- the	10:33:53
3	patent specification?	10:33:57
4	A Can you -- can you -- I'm not quite	10:33:59
5	understanding the question.	10:34:05
6	Q Yeah. So you said that the -- it doesn't	10:34:05
7	talk about modifications in -- actually, what I --	10:34:13
8	let me go back up.	10:34:20
9	You said that the patent specification has a	10:34:21
10	long list of modifications that can be added to	10:34:24
11	antisense oligonucleotides?	10:34:26
12	A The specifications talk about modifications	10:34:28
13	that can be incorporated into the bases in the -- in	10:34:30
14	the other -- in the ends. I should say the claim, I	10:34:36
15	guess it -- in terms of modifications, it says	10:34:41
16	uracils are thymines and that the antisense	10:34:43
17	oligonucleotide is a morpholino antisense	10:34:47
18	oligonucleotide.	10:34:51
19	So -- but beyond those modifications, it	10:34:52
20	doesn't talk about the other modifications other	10:34:53
21	than in the -- and a pharmaceutically acceptable	10:34:55
22	salt.	10:35:01
23	Q Okay. And so -- so what words, then, are	10:35:01
24	you pointing to as supporting your view that the	10:35:09
25	claim allows for modified bases?	10:35:12

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1	A	The specifications in the -- in -- in the	10:35:16
2		patent talk of -- talk about all the modifications	10:35:20
3		that can be used to make an antisense	10:35:23
4		oligonucleotide.	10:35:31
5	Q	And those modifications were known in the	10:35:31
6		field prior to the '851 patent?	10:35:34
7	A	Yes. Many of them were, yep. I mean,	10:35:36
8		that's why they were described.	10:35:40
9	Q	And -- and so again, going back to my -- my	10:35:43
10		first question, then -- then what words do you rely	10:35:49
11		on in the text of claim 1 as indicating to you that	10:35:52
12		you can use modified bases?	10:35:59
13	A	They don't say you can't use modified bases.	10:36:00
14		They say that it can be anti- -- I think if you read	10:36:09
15		this, the claim's saying that it can be this; and	10:36:10
16		then you read the patent, it says that -- that	10:36:12
17		further specifies what an antisense oligonucleotide	10:36:15
18		can be. Together, those two things teach us that	10:36:18
19		they can be modified.	10:36:23
20	Q	What can be modified?	10:36:24
21	A	All the different modifications that are	10:36:26
22		specified.	10:36:29
23	Q	All right. Let me see if I can clarify	10:36:31
24		here.	10:36:33
25		So are you focusing on the word "bases"?	10:36:33

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1	March of 2023. Your expert reports came out last	10:50:58
2	fall in -- later in 2023.	10:51:02
3	And so my question is: Prior to your expert	10:51:04
4	reports when you were just commenting on claim	10:51:07
5	construction issues, at that time, did you see the	10:51:09
6	word "bases" as encompassing these modified bases?	10:51:13
7	MS. LO: Same objections.	10:51:19
8	THE WITNESS: Yeah, I would say that I did	10:51:20
9	see that. I don't recall whether we had put it in	10:51:24
10	here or not.	10:51:27
11	BY MR. FRAZIER:	10:51:28
12	Q So it's something that -- that you believe	10:51:30
13	was in your head, but you didn't include it in the	10:51:34
14	declaration, to the best of your recollection?	10:51:37
15	A I don't have to say it's in my head. I	10:51:39
16	mean, this is common -- you know, if you're an	10:51:41
17	expert in this field, this is my job. This is --	10:51:43
18	you know, I'm very well aware that bases are	10:51:45
19	modified. The patents talks about modified bases.	10:51:47
20	It's very standard to understand that bases can be	10:51:51
21	modified.	10:51:54
22	Q All right. Another of the opinions in	10:51:58
23	your -- going back to Exhibit 101 -- is that	10:52:00
24	morpholino allows for different chemical linkages;	10:52:08
25	is that correct?	10:52:16

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1	A Yes. Which part are you asking me? 102.	10:52:16
2	Q 101.	10:52:23
3	A Okay, 101.	10:52:24
4	Q And -- and same questions there. If you	10:52:36
5	look at claim 1 of the '851 patent, is there	10:52:39
6	anything in claim 1 of the '851 patent that tells	10:52:46
7	you that a morpholino antisense oligonucleotide can	10:52:51
8	have linkages other than phosphoramidite?	10:53:01
9	A It doesn't say they are phosphoramidite, so	10:53:07
10	I -- it only says that they're morpholino. So it	10:53:11
11	doesn't tell you whether -- what -- what the	10:53:14
12	linkages are.	10:53:18
13	Q All right. And if you go back to	10:53:20
14	Exhibit 104, which was your claim construction	10:53:22
15	declaration, do you see -- if you turn to page 39	10:53:25
16	and 40, so you cite to a review article by Summerton	10:53:43
17	and Weller from 1997 which, you say, describes the	10:53:57
18	design and preparation of morpholinos and notes the	10:54:07
19	structure of morpholino oligomers includes uracil	10:54:10
20	bases. Do you see that?	10:54:10
21	A In paragraph 111?	10:54:12
22	Q Yes.	10:54:14
23	A Yeah. So they've labeled that figure as --	10:54:15
24	yes.	10:54:18
25	Q All right. And that's -- that's the figure	10:54:20

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1	that you relied on in your declaration to the court	10:54:20
2	in connection with claim construction for the	10:54:24
3	meaning of the word "morpholino," correct?	10:54:26
4	MS. LO: Objection to form.	10:54:30
5	Mischaracterizes.	10:54:32
6	THE WITNESS: I'd just add that -- that this	10:54:33
7	was just added here to show the -- the structure of	10:54:36
8	the morpholino -- of the PMO ring.	10:54:40
9	BY MR. FRAZIER:	10:54:44
10	Q Okay. And it shows -- it shows the linkage	10:54:44
11	as a phosphoramidite linkage, right?	10:54:47
12	A It shows the linkage as a phosphoramidite,	10:54:51
13	but that -- the morpholino descriptor in the figure	10:54:57
14	is clearly to the -- to the -- to the sugar	10:55:01
15	structure, to the HEX-S ring. It doesn't seem to	10:55:03
16	call out specifically what the linkage is in this	10:55:08
17	figure.	10:55:11
18	Q It specifically depicts the linkage as a	10:55:11
19	phosphoramidite linkage, right?	10:55:14
20	A It does, but I think the label is	10:55:17
21	referring -- the label in this case was not	10:55:19
22	distinguishing that linkage. It was just -- it was	10:55:21
23	referring to the -- the morpholino, obviously,	10:55:26
24	because it's not labeled as a PMO. It's -- so	10:55:28
25	it's -- it's saying that it's -- it's -- this is	10:55:31

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1	just a description of what the morpholino sugar	10:55:33
2	looks like.	10:55:38
3	Q That's your testimony today?	10:55:38
4	A I mean, that's what it -- I mean, that's	10:55:41
5	what it -- that's what it's labeled as, right? I	10:55:45
6	mean, there's other -- I think we refer to other	10:55:48
7	things in my expert report, where Summerton also	10:55:49
8	lists -- is more focused on the linkages and shows	10:55:52
9	the different linkages that can be used.	10:55:57
10	In this case, he is clearly showing the	10:56:00
11	morpholino -- or pointing out the morpholino.	10:56:03
12	Q Right. And you say that -- in -- in your	10:56:07
13	declaration, sworn testimony to the court, you said	10:56:08
14	that the Summerton article notes the structure of	10:56:12
15	morpholino oligomers includes uracil bases, right?	10:56:16
16	A Well, I say that, in that paper, they	10:56:22
17	describe the design and preparation of morpholinos	10:56:24
18	and that the structure of morpholino oligomers	
19	includes uracil --	
20	(Reporter clarification.)	09:53:16
21	THE WITNESS: Yeah.	10:56:35
22	So I think here, I refer to the article	10:56:36
23	where they describe the morpholino oligomers, right?	10:56:42
24	And then I show something from that article. I	10:56:50
25	don't show all the -- I don't show all the figures	10:56:53

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1	in the article.	10:56:56
2	This particular figure is labeled pointing	10:56:56
3	out the -- referring to the morpholino.	10:57:00
4	BY MR. FRAZIER:	10:57:03
5	Q And it's referring to the morpholino oligo,	10:57:03
6	right?	10:57:07
7	A Well, it has two morpholino structures	10:57:07
8	there. So technically, then it becomes an oligo.	10:57:11
9	Q Only if they're linked, right?	10:57:16
10	A Well, because there's two shown here, the	10:57:17
11	morpholino.	10:57:20
12	Q It's -- the thing that makes it a morpholino	10:57:21
13	oligo is the fact that you have two bases that are	10:57:24
14	linked, right? That's what an oligo is?	10:57:27
15	A But it's not describing that it's a	10:57:29
16	phosphoramidite. It's only showing two morpholinos.	10:57:34
17	So it's saying it's a morpholino, that that's the	10:57:39
18	structure of the ribose, that it's a morpholino.	10:57:43
19	Q Okay. I understand you have things you want	10:57:46
20	to say. I just need you to answer my questions.	10:57:48
21	All right?	
22	The question I said is: The thing that	10:57:50
23	makes this a picture of a morpholino oligo is that	10:57:52
24	it is two bases that are linked, right?	10:57:56
25	MS. LO: Objection. Argumentative.	10:57:58

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1	that in claim 1?	10:59:12
2	Q Let me -- let me go about it this way.	10:59:14
3	If you turn to paragraph 57 in Exhibit 101,	10:59:16
4	which is your report, just to orient you.	10:59:21
5	A Page 57?	10:59:31
6	Q That's paragraph 57 --	10:59:33
7	A Paragraph 57?	10:59:36
8	Q -- on page 23.	10:59:37
9	A Okay.	10:59:47
10	Q And you say that: As of 2005 -- reading	10:59:48
11	from paragraph 57 -- it was known that many	10:59:53
12	different chemical moieties could be added to either	10:59:56
13	the 5' or 3' or both ends of the antisense	11:00:01
14	oligonucleotides.	
15	Do you see that?	11:00:06
16	A I see that, yes.	11:00:06
17	Q And the potential for additional moieties to	11:00:08
18	be added or conjugated to the 5' or 3' end of the	11:00:16
19	antisense oligo is part of the basis for your	11:00:22
20	opinion that the claims lack enablement and written	11:00:25
21	description, correct?	11:00:30
22	MS. LO: Objection to form.	11:00:34
23	THE WITNESS: The -- the -- the	11:00:35
24	specification describes the different -- that the 5'	11:00:39
25	and 3' end could be modified.	11:00:44

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1	BY MR. FRAZIER:	11:00:46
2	Q You understand that what matters for	11:00:46
3	purposes of enablement is the claim?	11:00:50
4	A Yes. The claim is important -- yeah, right.	11:00:55
5	Q Okay. And so again, going back to claim 1	11:01:00
6	of the '851 patent, what is it in claim 1 that you	11:01:05
7	are relying on as indicating that you can add	11:01:08
8	moieties to the 5' or 3' end of the antisense oligo?	11:01:14
9	A Well, the -- the claim doesn't talk about	11:01:19
10	what the end is. So you don't know what the end is,	11:01:22
11	right? So the person would need to refer to the	11:01:26
12	specifications to think about what they're going to	11:01:31
13	put on the ends of the oligonucleotide. And	11:01:33
14	furthermore, the specification talks about how	11:01:40
15	things can be put on the end.	11:01:43
16	Q And one of the options is to put nothing on	11:01:44
17	the end, correct?	11:01:47
18	A Put -- to put the OH on the end.	11:01:48
19	Q Right. Which is just what would naturally	11:01:51
20	be there?	11:01:55
21	A Depending on how you synthesized.	11:01:55
22	Q All right. Going back to the modified	11:02:02
23	bases, you have not provided examples of modified	11:02:32
24	bases that would prevent the antisense	11:02:38
25	oligonucleotides of claim 1 from skipping, correct?	11:02:46

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1	A Are you -- are you talking about examples	11:02:50
2	that -- of how modifications change the activity of	11:02:57
3	the oligonucleotide?	11:03:03
4	Q No. I'm -- I'm asking if you have any	11:03:04
5	evidence that you're providing in this case of	11:03:06
6	oligonucleotides that are within the scope of	11:03:12
7	claim 1 that have been modified and, as a result of	11:03:16
8	that modification, they no longer have skipping	11:03:19
9	activity.	11:03:21
10	A I see what you're asking me.	11:03:22
11	Well, [REDACTED]	11:03:24
12	[REDACTED]	11:03:28
13	[REDACTED]	11:03:31
14	[REDACTED]	11:03:38
15	Matsuo had shown that there was ENAs that	11:03:39
16	did not have activity in that region, I believe in	11:03:42
17	that same region. So I would say that there was a	11:03:44
18	lot of evidence that modifications affected the	11:03:47
19	activity.	11:03:50
20	Q All right. My question has -- was modified	11:03:50
21	bases. Let's focus on modified bases.	11:03:52
22	A Oh, I see what you're saying.	11:03:56
23	Q Right. And -- and the example you just gave	11:03:56
24	didn't have to do with --	11:03:57
25	A Okay.	

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1	Q -- modified bases --	11:03:58
2	A Okay.	11:03:59
3	Q -- right?	11:03:59
4	A Right.	
5	Q Okay. It's important that we not talk at	11:04:00
6	the same time. So just -- just let me finish, and I	11:04:02
7	will wait for you if you can wait for me.	11:04:04
8	A No problem.	11:04:07
9	Q Okay. So my question then, going back:	11:04:08
10	With respect to modified bases, you have not cited	11:04:16
11	any evidence of an oligo within the scope of claim 1	11:04:20
12	that, as a result of modifying its bases, no longer	11:04:27
13	has skipping activity, correct?	11:04:31
14	A I would say, yeah, nobody had tested any	11:04:33
15	oligos at that time on how the modified bases had --	11:04:39
16	had affected the activity. [REDACTED]	11:04:47
17	[REDACTED]	11:04:51
18	[REDACTED]	11:04:55
19	Q Right. And you have not done any testing of	11:04:57
20	your own to show that modifying bases will result in	11:05:00
21	the loss of the ability to skip for an	11:05:08
22	oligonucleotide within the scope of claim 1, right?	11:05:11
23	A I would say within the scope of claim 1, not	11:05:14
24	specifically. But I am certainly well aware of how	11:05:16
25	base modifications can affect the activity, and this	11:05:19

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1	was well-known before even that.	11:05:22
2	I cite in my expert report, as early as 1997	11:05:26
3	there was a wealth of information on how the	11:05:29
4	modified bases did affect -- could -- could affect	11:05:30
5	the activity of the antisense oligonucleotides. And	11:05:34
6	so there is definitely good reason for them to put	11:05:40
7	that specification in there, that -- that	11:05:43
8	modifications can affect the activity.	11:05:45
9	Q All right. But you did do a series of tests	11:05:48
10	in this case, correct?	11:05:51
11	A Are you asking me about the CERI?	11:05:53
12	Q I am.	11:06:00
13	A The CERI, yes.	11:06:01
14	Q All right. And -- and in any of those	11:06:01
15	tests, did you choose to make oligonucleotides with	11:06:02
16	modified bases using the modifications that you've	11:06:07
17	identified out of the specification of the '851	11:06:10
18	patent?	11:06:13
19	A I -- when we -- when I designed the CERI	11:06:13
20	experiments, the claim construction was different.	11:06:16
21	And so we were really focusing on testing the -- the	11:06:19
22	antisense oligonucleotides under the former claim	11:06:22
23	construction. We were not focused on testing the --	11:06:24
24	the base modifications. We had -- you know, it	11:06:29
25	wasn't really our -- to, you know -- yeah.	11:06:36

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1	Q And, Doctor, respectfully, that's not true	11:06:38
2	at all, right? What you just said isn't true.	11:06:41
3	MS. LO: Objection. Argumentative.	11:06:44
4	BY MR. FRAZIER:	11:06:47
5	Q There was no claim construction at the time	11:06:47
6	you designed the CERI studies, right?	11:06:48
7	A Oh, I see what you're saying.	11:06:51
8	Well, the way that we were interpreting --	11:06:52
9	the way that I was interpreting the claim when I	11:06:54
10	first got involved in designing the CERI, right? So	11:06:57
11	I misspoke in terms of -- I know the claim	11:07:01
12	construction is a legal term. But the way that I	11:07:03
13	construed the claims of that when I was designing	11:07:06
14	the CERI reports were --	11:07:08
15	Q All right.	
16	A -- such that the -- we were -- I was testing	11:07:10
17	sort of the breadth of the -- the -- kind of the	11:07:12
18	four corners there.	11:07:14
19	Q All right. So you designed a series of	11:07:18
20	tests of oligonucleotides attempting to re-create	11:07:22
21	the conditions of the '851 patent in order to see	11:07:29
22	what the activity of oligonucleotides recited in the	11:07:32
23	patent would be, correct?	11:07:37
24	A We -- I tested some antisense	11:07:41
25	oligonucleotides that were recited in the patent and	11:07:49

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1	others that were not recited in the patent.	11:07:51
2	Q And the purpose of those tests was to	11:07:59
3	demonstrate that the '851 patent was not enabled or	11:08:05
4	not adequately described?	11:08:08
5	MS. LO: Objection to form.	11:08:12
6	THE WITNESS: I was interested in testing	11:08:14
7	the activity of antisense oligonucleotides across	11:08:16
8	the broad genus.	11:08:19
9	BY MR. FRAZIER:	11:08:21
10	Q You didn't have a -- a particular goal of --	11:08:21
11	of demonstrating that the -- the patent would be	11:08:25
12	invalid?	11:08:27
13	A I chose things across the -- the broad genus	11:08:30
14	of the -- of the patent focused on, I thought --	11:08:34
15	what I thought was a pretty diverse representation,	11:08:37
16	a good representation of the different -- the -- the	11:08:41
17	different species that would be represented through	11:08:47
18	that very broad claim.	11:08:51
19	Q But you didn't choose to test any oligos	11:08:53
20	with modified bases, correct?	11:08:57
21	A At the time, we were really focused on	11:09:00
22	the -- on the base, on the -- you know, on the	11:09:04
23	breadth of the sequences that were.	11:09:07
24	Q All right. And you could have chosen to	11:09:12
25	test oligos with modified bases, correct?	11:09:14

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1 A We could have. I mean, that's still not 11:09:17
2 straightforward to test modifications, in particular 11:09:23
3 with the PMOs. It's -- but we could have. We were 11:09:28
4 just not focused on -- on that. I wasn't focused on 11:09:35
5 that. 11:09:39

6 Q All right. And you, likewise, did not test 11:09:46
7 in your CERI testing any oligos that are morpholinos 11:09:47
8 that have other than phosphoramidate linkages, 11:09:56
9 correct? 11:10:02

10 A I did not. Because I was -- you know, 11:10:02
11 it's -- like I said, it's very difficult to get 11:10:03
12 those manufactured. 11:10:05

13 Q And that's because the generally accepted 11:10:10
14 linkage is the phosphoramidite linkage? 11:10:16

15 MS. LO: Objection to form. 11:10:20

16 THE WITNESS: I would say that -- that 11:10:22
17 their -- the PMOs are -- are -- are the 11:10:22
18 morpholino -- with the morpholino chemistry are 11:10:26
19 difficult to synthesize. So we certainly did not 11:10:29
20 test everything that could have been -- been tested. 11:10:32

21 BY MR. FRAZIER: 11:10:36

22 Q But this is an important case, right? And 11:10:36
23 you could have tested it if you wanted to? 11:10:39

24 A At the time when we -- when I designed to 11:10:42
25 test this, I tested across the broad genus. And we 11:10:44

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1	did definitely find that there were oligonucleotides	11:10:51
2	that were not active at that time.	11:10:53
3	Q Let me just repeat my question.	11:10:57
4	This is an important case, and you could	11:10:58
5	have tested, if you wanted to, PMOs with linkages --	11:11:00
6	or morpholinos with linkages other than	11:11:05
7	phosphoramidite, right?	11:11:10
8	A You know, the time that it takes to find	11:11:12
9	somebody that could make them and -- and make them	11:11:17
10	within the context of the case might have been	11:11:20
11	difficult. But we -- like I said, we were focused	11:11:23
12	on testing the -- the -- the breadth of the genus at	11:11:26
13	that time.	11:11:39
14	Q You mentioned the Summerton 1997 article	11:11:39
15	that was cited in your claim construction	11:12:39
16	declaration, right?	11:12:43
17	A Yes.	11:12:45
18	MR. FRAZIER: All right. Let's mark as	11:12:50
19	Exhibit Hastings 105 the document with Bates	11:13:38
20	No. SRPT-VYDS-229432 through 445, which is an	11:13:42
21	article by Summerton and Weller from 1997 titled	11:13:54
22	"Morpholino Antisense Oligomers: Design,	11:14:00
23	Preparation, and Properties."	11:14:04
24	(Hastings Exhibit 105 was marked for	
25	identification.)	

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1	BY MR. FRAZIER:	11:14:55
2	Q All right. And if you also turn to	11:14:55
3	Exhibit 102, which is your supplemental reply expert	11:14:59
4	report; and for reference, you can look at	11:15:03
5	paragraph 19.	11:15:12
6	And paragraph 19 refers to this Summerton	11:15:27
7	1997 article that we marked as Exhibit 105. And you	11:15:31
8	note that it refers to additional linkages --	11:15:37
9	intersubunit linkages that in 1997 could have been	11:15:45
10	used, correct?	11:15:49
11	A Correct.	11:15:51
12	Q And -- but if you turn in the document to	11:15:52
13	paragraph -- or to page 189 under the heading	11:15:56
14	"Intersubunit Linkage" --	11:16:02
15	A Okay.	11:16:04
16	Q -- do you see under that heading it says:	11:16:05
17	We have assessed a substantial number of	11:16:11
18	intersubunit linkage types. Then it tells the	11:16:14
19	chemical names of those types.	11:16:19
20	And then it says: Although morpholino	11:16:20
21	oligos containing a number of such linkages provide	11:16:24
22	effective binding to targeted genetic sequences,	11:16:28
23	consideration of cost and ease of synthesis,	
24	chemical stability, aqueous solubility, and affinity	11:16:33
25	and homogeneity of bindings to the RNA led us to	11:16:37

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

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1	Yeah, I see that sentence.	12:54:35
2	Q Okay. And does that reflect the idea that	12:54:36
3	by 2002, morpholino as a term was becoming	12:54:41
4	associated with the phosphorodiamidate linkage	12:54:45
5	rather than other types of linkages?	12:54:52
6	A I wouldn't -- I would not agree with that	12:54:54
7	statement. I think that that -- she just had a --	12:54:56
8	she was just being somewhat casual about her --	12:55:00
9	her -- you know, I mean, she also refers to them as	12:55:05
10	oligos rather than oligonucleotides. So I think	12:55:08
11	that just the general sort of -- she -- I think	12:55:12
12	she's just being very casual about her definitions	12:55:13
13	here.	12:55:16
14	Q So do you cite any evidence in -- in your	12:55:17
15	reports suggesting that by 2002, there was not a	12:55:21
16	generally accepted understanding of what morpholinos	12:55:25
17	meant?	12:55:29
18	A I mean, a morpholino is what it is, right?	12:55:29
19	I mean, it's that -- it's that particular moiety.	12:55:34
20	Whether or not people want to shorten	12:55:38
21	oligonucleotide to oligo or PMO to morpholino is	12:55:41
22	something that definitely is -- like I said, it's	12:55:47
23	kind of jargon -- it's just jargony. It's probably	12:55:49
24	not appropriate necessarily for a scientific	12:55:51
25	publication because it introduces ambiguity.	12:55:53

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

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1 Q But in -- in terms of -- of in the context 12:55:56
2 of making oligonucleotides, morpholino 12:55:58
3 oligonucleotides, by 2005, the filing of the Wilton 12:56:03
4 '851 patent, that term morpholino generally was used 12:56:10
5 to mean a phosphorodiamidite linkage oligo, was it 12:56:16
6 not? 12:56:24

7 A I -- I couldn't agree with saying generally. 12:56:24
8 I mean, clearly people were using it to refer to 12:56:27
9 that. I think that people that were -- when you get 12:56:30
10 right down to synthesizing it, obviously you have -- 12:56:32
11 have to be more precise. 12:56:34

12 Q Let me go back briefly to the CERI studies 12:56:46
13 that we were looking at earlier. 12:56:51

14 And do you agree that in those studies, you 12:57:15
15 did not test any oligos within the scope of claim 1 12:57:18
16 of the '851 patent that were chemically linked to 12:57:23
17 any of the moieties or conjugates? 12:57:26

18 A Can you rephrase that question? 12:57:32

19 Q Yeah. 12:57:35

20 In the context of the CERI study and the 12:57:35
21 three reports that were generated from that work, 12:57:39
22 did you test any oligos covered by claim 1 of the 12:57:43
23 '851 patent that were linked to moieties or 12:57:47
24 conjugates? 12:57:51

25 A We -- we tested the PMOs and the 12:57:53

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

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1	2'-0-methyls. And are you asking about the 3'	12:58:01
2	modifications or --	12:58:07
3	Q Either.	12:58:07
4	A Right, we didn't test any of the 3' or 5'	12:58:07
5	modifications.	12:58:11
6	Q If you turn to paragraph 210 of Exhibit 101,	12:58:21
7	your opening report --	12:58:25
8	A Okay.	12:58:26
9	Q -- your opening --	12:58:27
10	A Paragraph 210?	12:58:28
11	Q Yes.	12:58:31
12	-- you have a footnote that goes along with	12:58:48
13	paragraph 210 that says: The CERI reports, which	12:58:51
14	tested only 29 antisense oligonucleotides out of the	12:58:54
15	tens of millions of antisense oligonucleotides	12:58:59
16	encompassed by the genus of the UWA patent claims,	12:59:03
17	took upwards of 14 months to complete.	12:59:07
18	Do you see that?	12:59:10
19	A I see that, yes.	12:59:10
20	Q All right. That's not accurate, is it?	12:59:14
21	A Can you be more specific?	12:59:15
22	Q Well, it's just not true that the CERI	12:59:16
23	studies took 14 months, right?	12:59:20
24	A I believe from the time we started to design	12:59:20
25	to get everything synthesized and protocol written	12:59:23

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

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1	and working with -- working to get the experiments	12:59:27
2	done, it was 14 months.	12:59:29
3	Q And if you -- you still have the reports in	12:59:31
4	front of you? If you look at the -- let's look at	12:59:37
5	Exhibit 106, which is the CERI 2'-O-methyl report.	12:59:40
6	A 104?	13:00:12
7	Q 106.	13:00:14
8	A Okay. Oh, that --	13:00:15
9	Q It has Bates No. 102 --	13:00:28
10	A I think it's this one. This one, right?	13:00:30
11	10 -- it says -- that's a 6, right?	13:00:34
12	Q And it's Bates No. 102924, at the bottom	13:00:37
13	right?	13:00:42
14	A Yep.	13:00:42
15	Q Okay. All right. And so this is --	13:00:43
16	Exhibit 106 is the report from the 2'-O-methyl	13:00:45
17	study, right?	13:00:56
18	A Yes.	13:00:57
19	Q And it has Study No. 0643 up in the top,	13:00:57
20	right?	13:01:01
21	A Correct.	13:01:03
22	Q And if you turn to page 926, which is page 1	13:01:03
23	of the document, you see there's a section next to	13:01:07
24	the heading 5, Period of Study?	13:01:11
25	A Yes.	13:01:14

Transcript of Michelle Hastings, Ph.D.
Conducted on September 13, 2024

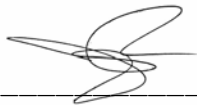
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C E R T I F I C A T E

I, JENNIFER L. BERNIER, a Certified
Shorthand Reporter, Registered Merit Reporter,
Certified Realtime Reporter, the officer before whom
the foregoing videotaped deposition was taken, do
hereby certify that the foregoing transcript is a
true and correct record of the testimony given; that
said testimony was taken by me stenographically and
thereafter reduced to typewriting under my
supervision; that reading and signing was requested;
and that I am neither counsel for or related to, nor
employed by any of the parties to this case and have
no interest, financial or otherwise, in its outcome.

IN WITNESS WHEREOF, I have hereunto set my
hand this September 18, 2024.

My commission expires May 31, 2025.



JENNIFER L. BERNIER, CSR, RMR, CRR

Exhibit 4

IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE

<hr/>)	
NIPPON SHINYAKU CO., LTD.,)	
Plaintiff,)	
)	
v.)	
)	C.A. No. 21-1015 (JH)
SAREPTA THERAPEUTICS, INC.,)	
Defendant.)	
<hr/>)	
SAREPTA THERAPEUTICS, INC. and)	
THE UNIVERSITY OF WESTERN)	
AUSTRALIA, Defendant and Counter-)	
Plaintiff)	
)	
v.)	
)	
NIPPON SHINYAKU CO., LTD. and)	
NS PHARMA, INC., Plaintiff and)	
Counter-Defendants.)	
<hr/>		

EXPERT REPORT OF DR. MICHELLE L. HASTINGS
REGARDING INVALIDITY OF THE UWA PATENTS

July 3, 2024



Michelle L. Hastings, Ph.D.

VII. TECHNOLOGICAL BACKGROUND

43. If called to testify at trial, I will be able to provide a tutorial to explain and provide information about antisense oligonucleotides, pre-mRNA splicing, and exon skipping in general, as well as the use of antisense oligonucleotides for the treatment of medical conditions, such as Duchenne Muscular Dystrophy. I have reviewed the technological background that Dr. Matthew J.A. Wood provided in his opening expert report and supplemental expert report that he submitted in this case. Wood Report ¶¶ 17-116; *see generally* Wood Suppl. Rpt. ¶¶ 11-86. *see also* Declaration of Matthew J.A. Wood submitted in Patent Interference No. 106,007 (“Wood Interference Declaration”) ¶¶ 13-119. I agree with his technological background and incorporate those paragraphs herein.

VIII. THE SCOPE OF THE ASSERTED CLAIMS FAR EXCEEDS THE DISCLOSURE IN THE SPECIFICATION

44. Even after the Court’s revised construction, it remains my opinion that the UWA Patent claims are drawn to a broad genus that encompasses a tremendous number of antisense oligonucleotide species. The claims of the UWA Patents are each directed to morpholino antisense oligonucleotides that induce exon 53 skipping, but permit extensive structural and functional variability across members of the genus.

45. The claimed morpholino antisense oligonucleotides do not need to be one exact length—they may range anywhere between “20 to 31 bases” in length (*i.e.*, twelve different possible lengths).

46. The claimed morpholino antisense oligonucleotides do not need to have one exact sequence of bases. As construed, their bases must (1) have “100% complementarity to consecutive bases of a target region of exon 53 throughout the entire length of the antisense oligonucleotide,” *see* D.I. 573; (2) comprise at least 12 consecutive bases of SEQ ID NO: 195; and (3) have thymine

bases instead of uracil bases.” For the ’851 Patent, the “target region” must also be “within nucleotides +23 to +69 of exon 53 of the human dystrophin pre-mRNA.” A wide variety of different morpholino antisense oligonucleotides satisfy all of the claimed structural requirements.

47. The number of species encompassed by the claims of the UWA Patents can be estimated using the design options allowed by the claims, such as the possible lengths (20 to 31 bases), the possible “target regions,” and the possible chemical variations allowed by the claims.

A. Claimed ASOs Bind to Dozens of Different “Target Regions”

48. As shown below, thirteen (13) different combinations of “at least 12 consecutive bases of SEQ ID NO: 195” are possible from SEQ ID No. 195’s 25-base-long sequence:

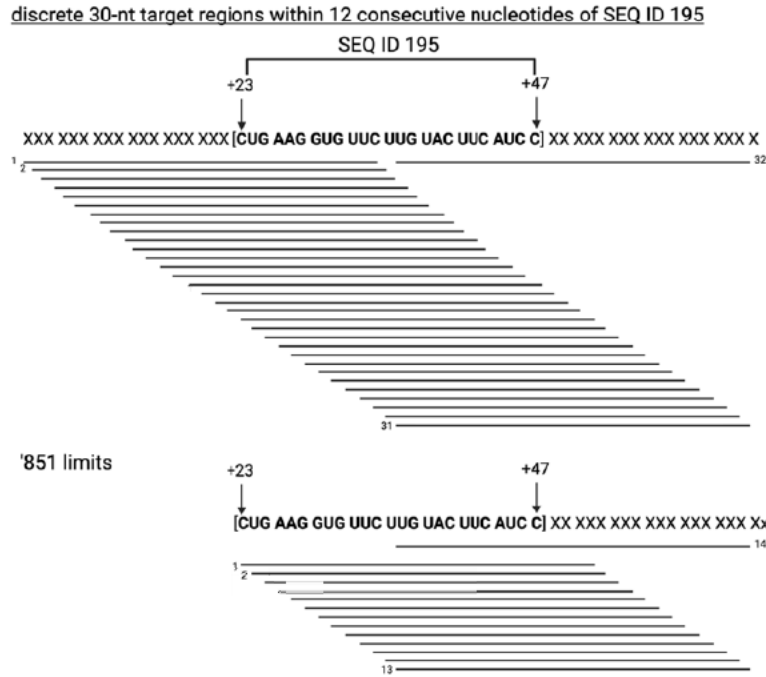
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C

Thus, there are multiple different “target regions” possible for each given length of antisense oligonucleotide (20 to 31 bases) that will still allow 100% complementary morpholino antisense oligonucleotides to comprise 12 consecutive bases of SEQ ID No. 195.

49. As indicated below, there will be a farthest 5’ “target region” and a farthest 3’ “target region” for each given antisense oligonucleotide length (20 to 31 bases), as well as multiple possible “target regions” in between:



Length	Farthest 5' Target	Farthest 3' Target	Discrete "Target Regions"
20mer	h53A(+15+34)	h53A(+36+55)	22
21mer	h53A(+14+34)	h53A(+36+56)	23
22mer	h53A(+13+34)	h53A(+36+57)	24
23mer	h53A(+12+34)	h53A(+36+58)	25
24mer	h53A(+11+34)	h53A(+36+59)	26
25mer	h53A(+10+34)	h53A(+36+60)	27
26mer	h53A(+9+34)	h53A(+36+61)	28
27mer	h53A(+8+34)	h53A(+36+62)	29
28mer	h53A(+7+34)	h53A(+36+63)	30
29mer	h53A(+6+34)	h53A(+36+64)	31
30mer	h53A(+5+34)	h53A(+36+65)	32
31mer	h53A(+4+34)	h53A(+36+66)	33
Total Discrete "Target Regions" ('590 & '827 Patent)			330



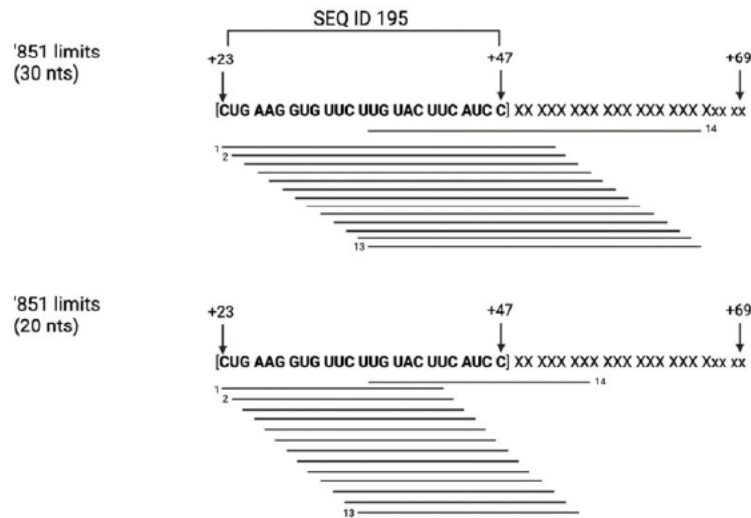
50. Because the '851 Patent additionally requires that the “target region” be “within nucleotides +23 to +69 of exon 53 of the human dystrophin pre-mRNA,” its “target regions” are instead as follows:

Length	Farthest 5' Target	Farthest 3' Target	Discrete “Target Regions”
20mer	h53A(+23+42)	h53A(+36+55)	14
21mer	h53A(+23+43)	h53A(+36+56)	14
22mer	h53A(+23+44)	h53A(+36+57)	14
23mer	h53A(+23+45)	h53A(+36+58)	14
24mer	h53A(+23+46)	h53A(+36+59)	14
25mer	h53A(+23+47)	h53A(+36+60)	14
26mer	h53A(+23+48)	h53A(+36+61)	14
27mer	h53A(+23+49)	h53A(+36+62)	14
28mer	h53A(+23+50)	h53A(+36+63)	14



29mer	h53A(+23+51)	h53A(+36+64)	14
30mer	h53A(+23+52)	h53A(+36+65)	14
31mer	h53A(+23+53)	h53A(+36+66)	14
Total Discrete “Target Regions” (’851 Patent)			168

discrete 30 and 20-nt target regions within 12 consecutive nucleotides of SEQ ID 195 within the region +23 to +69



B. Claimed ASOs May Have Various Chemical Structures

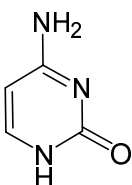
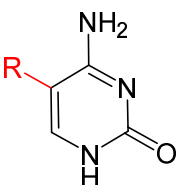
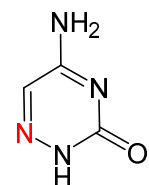
51. When designing 100% complementary antisense oligonucleotides to the possible “target regions,” the claims specify that certain chemical structures *must* be used. Each claimed antisense oligonucleotide must use “thymine bases instead of uracil bases.” And each claimed antisense oligonucleotide must use a “morpholino” chemical backbone (rather than other disclosed chemistries, such as 2’O-methyl phosphorothioate). Although the claims specify these structural aspects of the claimed ASOs, they allow for many other structural, chemical variations.

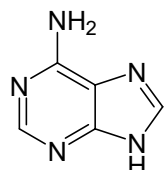
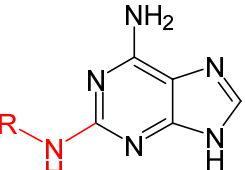
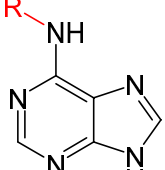
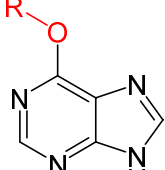
52. The UWA Patents’ specification itself describes a variety of chemical structures that a POSA may or may not choose to use when designing a claimed antisense oligonucleotide. The specification explains, for example, that “[o]ligonucleotides may also include nucleobase . . .

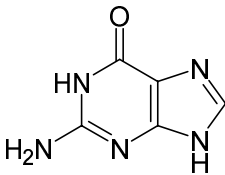
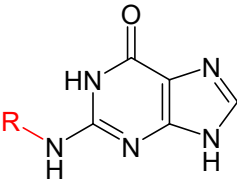
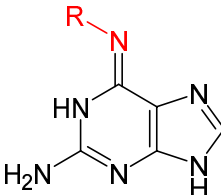
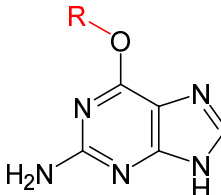
modifications or substitutions.” ’851 Patent at 27:37-42. As annotated below, there are five classes of modified nucleobases that the specification tells POSAs are possible:

Certain nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include [1] 5-substituted pyrimidines, [2] 6-azapyrimidines and [3] N-2, [4] N-6 and [5] O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

’851 Patent at 27:37-46. These classes involve modifying nucleobases at particular locations in their chemical structure. As illustrated below, a 5-substituted pyrimidine involves making a chemical substitution at the fifth-numbered position in a pyrimidine base (cytosine, uracil, and thymine), 6-azapyrimidines are pyridines with the sixth-numbered position substituted for a nitrogen atom, and N-2, N-6, and O-6 substituted purines involve substitutions at the second- or sixth-numbered positions of a purine base (adenine or guanine) involving chemical groups having, respectively, nitrogen or oxygen:

Unmodified/unsubstituted	“5-substituted pyrimidines”	“6-azapyrimidines”
 cytosine	 5-substituted cytosine	 6-azacytosine

Unmodified/unsubstituted	“N-2 substituted purines”	“N-6 substituted purines”	“O-6 substituted purines”
			

adenine	N-2 substituted adenine	N-6 substituted adenine	O-6 substituted adenine
 guanine	 N-2 substituted guanine	 N-6 substituted guanine	 O-6 substituted guanine

53. Within these classes, the specification identifies three examples of particular 5-substituted pyrimidines (5-propynyluracil and 5-propynylcytosine. 5-methylcytosine) and an example of a N-2 substituted purine (2-aminopropyladenine). Accordingly, given the claims' structural limitations,¹ a POSA would recognize at least nine different options for modified bases from this disclosure: four (4) options for pyrimidines (5-propynylcytosine; 5-methylcytosine; other 5-substituted pyrimidines; and 6-azapyrimidines), four (4) options for purines (2-aminopropyladenine; other N-2 substituted purines; N-6 substituted purines; and O-6 substituted purines) and having no modifications.

54. In 2014, Sarepta filed WO 2014/100714 A1 ("714 Publication") describing research conducted on exon 53-targeted PMOs. In that application, Sarepta's scientists included a highly similar instruction regarding how nucleobase "modifications or substitutions" could be used:

In some embodiments, the antisense oligonucleotides contain base modifications or substitutions. For example, certain nucleo-bases may be selected to increase the binding affinity of the antisense oligonucleotides described herein. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted

¹ I am not counting "5-propynyluracil" as an option because of the claim limitation "wherein uracil bases are thymine bases."



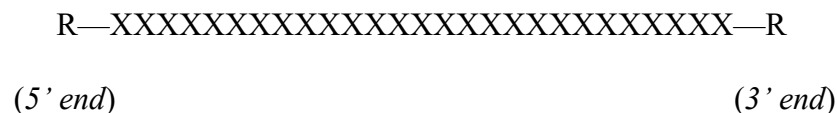
purines, including 2-aminopropyladenine, 5-propynyluracil, 5-propynylcytosine and 2, 6-diaminopurine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2. C, and may be incorporated into the antisense oligonucleotides described herein. In one embodiment, at least one pyrimidine base of the oligonucleotide comprises a 5-substituted pyrimidine base, wherein the pyrimidine base is selected from the group consisting of cytosine, thymine and uracil. In one embodiment, the 5-substituted pyrimidine base is 5-methylcytosine. In another embodiment, at least one purine base of the oligonucleotide comprises an N-2, N-6 substituted purine base. In one embodiment, the N-2, N-6 substituted purine base is 2, 6-diaminopurine.

'714 Publication at 5:31-6:6. Sarepta's scientists also provided a list of nucleobase substitutions and modifications that could be used. *Id.* at 24:5-25:2. In doing so, they highlighted a prior art patent (U.S. 6,683,173) that disclosed "[e]xamples of derivatives of Super A, Super G, and Super T." *Id.* at 24:15-17. This further supports my opinion that, between the state of the art and the specification's explicit instructions, a POSA would readily understand that claimed morpholino ASOs may have nucleotide modifications and substitutions at the time of the invention.

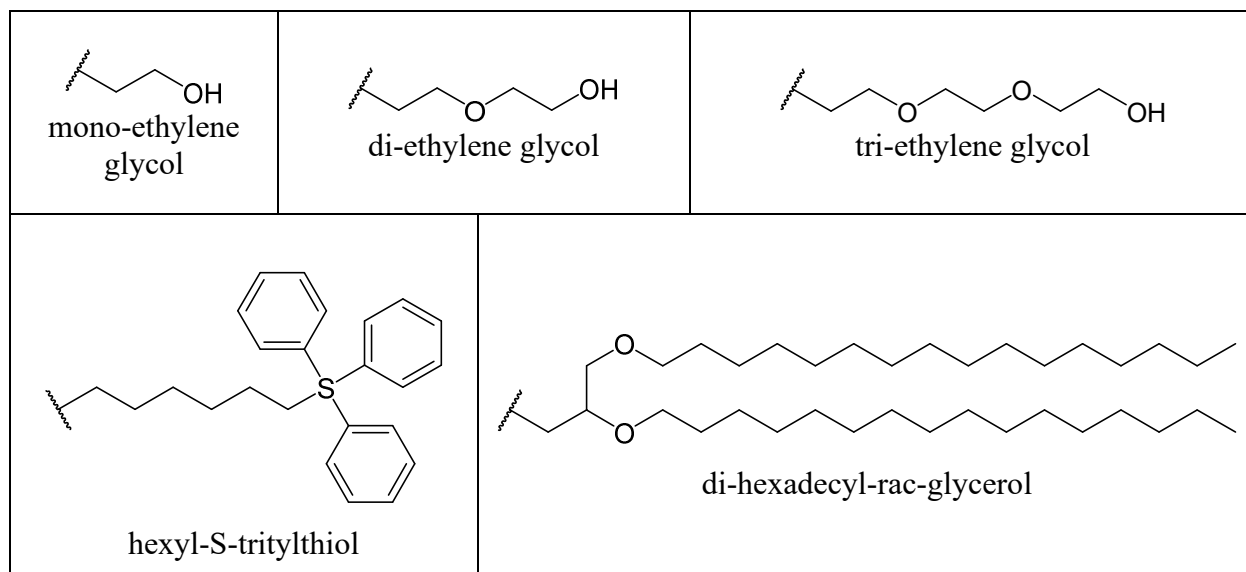
55. According to the UWA Patents' specification, "[a]nother modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates." '851 Patent at 27:47-59. As annotated below, there are at least nine classes and subclasses of moieties that the specification tells POSAs are possible:

Such moieties include but are not limited to [1] lipid moieties such as [2] a cholesterol moiety, cholic acid, [3] a thioether, e.g., hexyl-S-tritylthiol, [4] a thiocholesterol, [5] an aliphatic chain, e.g., dodecandiol or undecyl residues, [6] a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, [7] a polyamine or [8] a polyethylene glycol chain, or adamantane acetic acid, [9] a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

Id. As illustrated below, these classes involve modifying ASOs by performing a chemical modification at either the 5' or 3' (or both) ends of the antisense oligonucleotides:



The corresponding chemical moieties (only a few of which are depicted below) would be conjugated at an “R—” location (squiggly line below):



56. The specification identifies particular examples of moieties, such as cholic acid, hexyl-S-tritylthiol, dodecandiol residues, undecyl residues, di-hexadecyl-rac-glycerol, triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, adamantane acetic acid, octadecylamine and hexylamino-carbonyl-oxycholesterol. Accordingly, given the claims’ structural limitations, a POSA would recognize at least nineteen different options for chemical moieties from this disclosure: twelve (12) options for lipid moieties (a cholesterol moiety; cholic acid, hexyl-S-tritylthiol; another thioether; a thiocholesterol, dodecandiol residues; undecyl residues; another aliphatic chain; di-hexadecyl-rac-glycerol; triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate; another phospholipid; and another lipid moiety), as well as a polyamine, a polyethylene glycol chain, adamantane acetic acid; a palmityl moiety; an octadecylamine moiety; a hexylamino-carbonyl-oxycholesterol moiety; and having no moiety.

57. As of 2005, it was known that many different chemical moieties could be added to either the 5’ or 3’ or both ends of the antisense oligonucleotides. For example, it was demonstrated

as early as 1984 that attaching moieties at the end of oligonucleotides can influence their activity, motivating scientists to explore these types of modifications for oligonucleotide discovery.²

58. As an example, in the years preceding and following the time of the invention, a group of researchers (including some at Sarepta's predecessor, AVI Biopharma), published multiple articles discussing certain chemical moieties that had been used with PMOs.³ These articles discuss the use of fluorescein and other fluorescent moieties, arginine and fluorine-rich peptides, as well as using different moieties on each end of a PMO. Swenson et al. (2009) also exemplifies how implementing different moieties (even highly-similar moieties) may or may not affect a PMO's biological activity. Swenson et al. examined numerous moieties in the class of peptide moieties having various combinations of amino acids and peptide lengths. An antiviral PMO "was conjugated at either the 5' or the 3' terminus with various peptides bearing arginine (R), ornithine (O), methylated ornithine [O(Me)₂] or histidine (H) or was chemically modified at multiple positions along the PMO backbone with the positively charged piperazine groups," and they determined that only certain moieties enhanced the PMO's biological activity. Swenson (2009) at 2094. For example, although they reported that "conjugation with arginine-rich peptides generally enhanced the antiviral effect," they found that highly-similar "peptides containing less than eight arginine residues failed to enhance the antiviral activity relative to that achieved with

² Asseline et al., *Oligodeoxynucleotides covalently linked to intercalating dyes as base sequence-specific ligands. Influence of dye attachment site*, *The EMBO J.*, 3:795-800 (1984).

³ Neuman et al., *Antisense Morpholino-Oligomers Directed against the 5' End of the Genome Inhibit Coronavirus Proliferation and Growth*, *J. Virology*, 78:5891-5899 (2004); Neuman et al., *Inhibition, Escape, and Attenuated Growth of Severe Acute Respiratory Syndrome Coronavirus Treated with Antisense Morpholino Oligomers*, *J. Virology*, 79:9665-9676 (2005); Lebleu et al., *Cell penetrating peptide conjugates of steric block oligonucleotides*, *Advanced Drug Delivery Reviews* 60:517-529 (2008); Swenson et al., *Chemical Modifications of Antisense Morpholino Oligomers Enhance Their Efficacy against Ebola Virus Infection*, *Antimicrobial Agents & Chemotherapy*, 53:2089-2099 (2009).

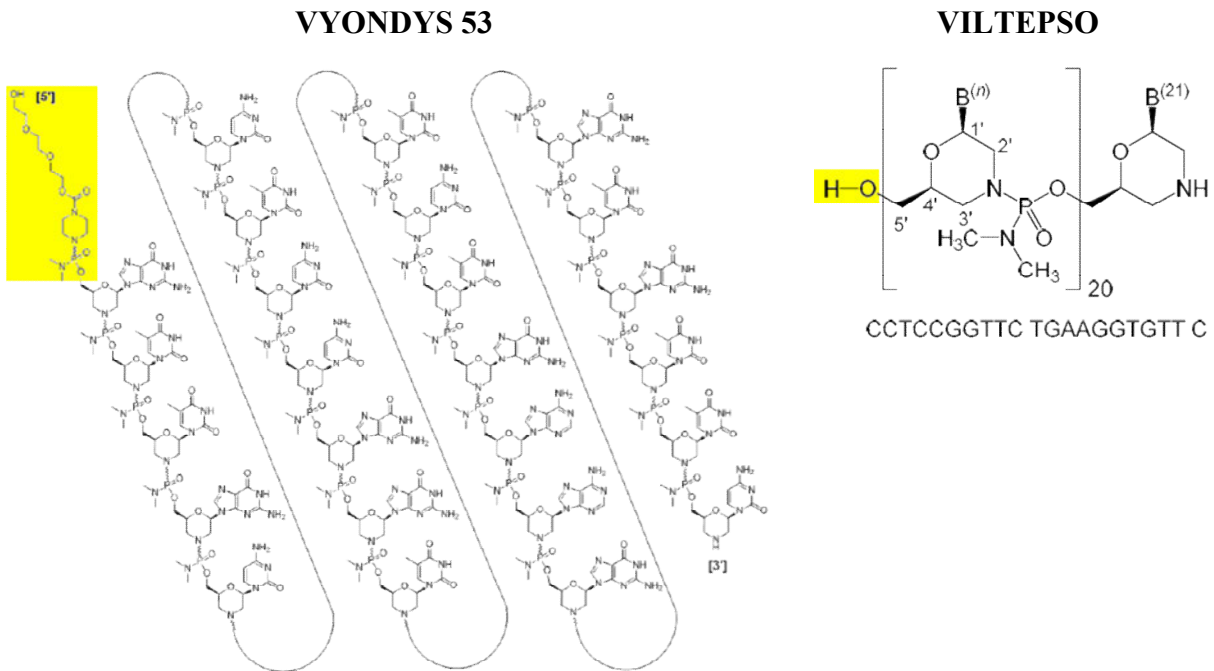
untagged PMO.” *Id.* Similar considerations are applicable to each broad class of moieties disclosed in the UWA Patents’ specification.

59. Additionally, in Sarepta’s 2014 ’714 Publication describing research conducted on exon 53-targeted PMOs, Sarepta’s scientists included a highly similar instruction regarding how chemical moieties could be used:

In one embodiment, another modification of the antisense oligonucleotides involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

’714 Publication at 25:3-10.

60. Thus, between the state of the art and the specification’s explicit instructions, a POSA would readily understand that claimed morpholino ASOs may have chemical moieties. In fact, I have been informed by counsel and understand that Sarepta affirmatively argues that both VYONDYS 53 (which has a chemical moiety including a TEG group linked to its 5’ end) and VILTEPSO (which has an OH group at its 5’ end) fall with the scope of the UWA Patents’ claims.

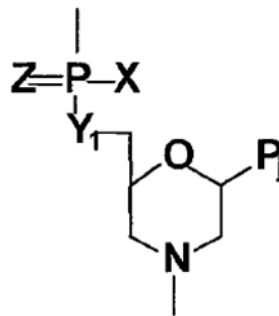


The UWA Patents' specification does not describe the chemical structure of the 5' moiety used with VYONDYS 53—a tri-ethylene glycol chain paired with the additional chemistry shown above. The closest the specification comes is generically referencing “a polyethylene glycol chain.”

61. As another example of chemical variations, I agree with Dr. Wood's conclusion that the term “morpholino” is broader than just a *phosphorodiamidate* morpholino oligomer (“PMO”) and encompasses ASOs with different types of morpholino intersubunit linkages. Wood Suppl. Rpt. ¶¶ 82-85. The '851 Patent specification only uses the term “morpholino” in table legends (referencing “other antisense chemistries such as . . . morpholinos”), and does not define it.

62. Sarepta has described morpholinos as any “oligonucleotide analog composed of morpholino subunit structures, where (i) the structures are linked together by phosphorus-containing linkages, one to three atoms long, preferably two atoms long, and preferably uncharged or cationic, joining the morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent

subunit, and (ii) each morpholino ring bears a purine or pyrimidine base-pairing moiety effective to bind, by base specific hydrogen bonding, to a base in a polynucleotide.” SRPT-VYDS-0228276 at 294-296. Accordingly, the only structural features required to meet the limitation “morpholino antisense oligonucleotide” is (1) a nucleotide base linked to a morpholino ring and (2) an antinucleotide linkage comprising phosphorus. *See id.* Sarepta provided the following exemplary general structure of an antisense morpholino subunit:



where: “Y₁ is -O-, -S-, -NH-, or -CH₃- [four possibilities], Z is O or S [two possibilities], P_j is a purine or pyrimidine base-pairing moiety effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide; and X is fluoro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted thioalkoxy, amino, optionally substituted alkylamino, or optionally substituted heterocyclyl [seven possibilities].” *Id.* at 283. As examples, phosphoroamidate morpholino oligomers (MO), phosphorodiamidate morpholino oligomers (PMO) and thiomorpholinos (TMO) would each meet this definition.⁴

63. The specification also informs POSAs that chemical modifications to ASOs may be mixed-and-matched: “It is not necessary f[o]r all positions in a given compound to be uniformly

⁴ See, e.g., Paul & Caruthers, *Synthesis of Backbone-Modified Morpholino Oligonucleotides Using Phosphoramidite Chemistry*, *Molecules*, 28:5380 (2023); Le et al., *Thiomorpholino oligonucleotides as a robust class of next generation platforms for alternate mRNA splicing*, *PNAS* 119(36) (2022).

modified [*i.e.*, modified in the same manner], and in fact ***more than one*** of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide.” *Id.* at 27:60-64 (emphasis added). As it explains, “[t]he present invention also includes antisense compounds that are chimeric compounds”—*i.e.*, “antisense molecules, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit.” *Id.* at 27:64-28:2. As to inter-nucleotide linkages (*e.g.*, the different forms of morpholino linkages), the specification explains that as few as “at least one” (or as many as “all”), “internucleotide bridging phosphate residues are modified phosphates.” *Id.* at 26:58-65 (“For example, every other one of the internucleotide bridging phosphate residues may be modified as described.”).

64. The 2014 ’714 Publication by Sarepta’s scientists regarding exon 53 PMOs includes largely the same instructions. They explain, for example, that “[i]t is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide.” ’714 Publication at 25:11-13; *see also id.* at 25:17-20 (explaining that “[t]he present invention also includes antisense compounds that are chimeric compounds”—*i.e.*, “antisense molecules, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit”). They likewise explained that as few as “at least one” (or as many as “all”), “internucleotide bridging phosphate residues are modified phosphates.” *Id.* at 22:23-31 (“For example, every other one of the internucleotide bridging phosphate residues may be modified as described.”).

65. Additionally, POSAs did—in fact—create chimeric morpholino ASOs in the years following June 2005.⁵ This further supports my opinion that a POSA would readily understand that claimed morpholino ASOs may be partially modified and/or include a combination of different morpholino linkages, nucleobase modifications and/or chemical moieties.

C. The UWA Patents’ Claims Encompass Vast Numbers of Structurally Distinct ASOs

66. As noted above, accounting for the possible lengths (20 to 31 bases), complementarity, and sequence limitations, the number of discrete possible “target regions” encompassed by the claims are either 168 (’851 Patent) or 330 (’590 and ’827 Patents). These numbers do *not* include antisense oligonucleotides targeting these regions that have the permitted structural variations, and thus only account for a small fraction of the total number of ASOs that meet the claims’ structural limitations.

67. Taken in context, however, a POSA at the time of the invention would consider even the number of discrete “target regions” for the ’851 Patent (168) and ’590 and ’827 Patents (330) to themselves be “vast.” To contextualize these figures, I compared them to testing that POSAs have actually performed on exon 53. Historically, when a group of POSAs—whether an academic institution or company—decided to examine exon 53 skipping, those groups tested relatively few exon 53-directed ASOs.⁶ For example:

⁵ Zhang et al., *Synthesis and properties of morpholino chimeric oligonucleotides*. Tetrahedron Letters 49:3570-3573 (2008); Paul & Caruthers, *Synthesis of Backbone-Modified Morpholino Oligonucleotides Using Phosphoramidite Chemistry*, Molecules, 28:5380 (2023)

⁶ As discussed more fully below, *infra* Section X, it is practically difficult to make and use many ASOs, particularly morpholino ASOs. If it were, as of 2005, actually “routine” to tests hundreds of ASOs (or more) in a research project, the contemporaneous publications describing (ostensibly) cutting-edge work would not be so limited in the amount of ASO testing described. In fact, even in recent years, POSAs continued to report that synthesis time for a single 20mer would take weeks. See, e.g., Li et al., *Fully automated fast-flow synthesis of antisense phosphorodiamidate morpholino oligomers*, Nature Comm’ns (2021) (“Changes to the chemistry of PMO synthesis are

- In WO 2004/083432 A1 (“Van Ommen”), researchers at Leiden University report testing only two (2) ASOs directed to exon 53, (*see* Van Ommen at tbl. 2);
- In WO 2004/048570 (“Matsuo”),⁷ researchers at Kobe University report testing only twelve (12) ASOs directed to exon 53, (*see* Matsuo at [0572]-[0573]);
- The UWA Patents report testing only eleven (11) ASOs directed to exon 53 (*see* ’851 Patent at tbl. 39 (SEQ ID No. 197 is “Not made yet”));
- In Popplewell (2009),⁸ researchers at Royal Holloway University report testing only twenty-three (23) morpholino ASOs directed to exon 53; (*see* Popplewell (2009) at fig. 1);
- In Popplewell (2010),⁹ those research report testing only twenty-four (24) morpholino ASOs directed to exon 53, (*see* Popplewell (2010) at 104, tbl. 1);
- In US 2010/0130591 A1 (“’591 Publication”), Sarepta researchers report testing only twenty-four (24) peptide conjugated morpholino ASOs directed to exon 53, (*see* ’591 Publication at [0293]);
- In WO 2010/050802 A2 (’802 Publication”), Prosensa researchers identify forty-eight (48) ASOs directed to exon 53, (*see* ’802 Publication at tbl. 6); and
- In WO 2014/100714 A1 (“’714 Publication”), other Sarepta researchers report testing only nine (9) morpholino ASOs directed to exon 53, (*see* ’714 Publication at Ex. 1).

As a stark outlier, the Nippon Shinyaku and NCNP inventors’ later work reports ***one hundred and twenty-three (123)*** ASOs that all are directed to exon 53, and provide test results for more than ***eighty (80)*** of those exon 53-directed ASOs (some in morpholino ASOs and others in 2’OMe ASOs). *See, e.g.*, ’092 Patent at cols. 44-46, figs. 1-19.

greatly needed to enable the rapid drug development” because “with therapeutic PMO sequences on the order of 20 residues, synthesis times are on the order of weeks”).

⁷ I have relied on US2007/0082861, which I understand is an English publication of WO2004/048570.

⁸ Popplewell, Linda, et al. “Design of Phosphorodiamidate Morpholino Oligomers (PMOs) for the Induction of Exon Skipping of the Human DMD Gene” (Popplewell 2009) (SRPT-VYDS-0008344).

⁹ Popplewell, Linda, et al., “Comparative analysis of antisense oligonucleotide sequences targeting exon 53 of the human DMD gene: Implications for future clinical trials” (Popplewell 2010) (SRPT-VYDS-0198789).



68. As of June 2005, the largest number of exon 53 ASOs reported by any group of POSAs as being tested was Matsuo’s twelve (12) ASOs. Thus, the ’851 Patent allows for *fourteen (14) times* more discrete “target regions” than the number of exon 53 ASOs any POSA had—at that time—ever evaluated at a given time. Likewise, the ’590 and ’827 Patents allow for *twenty-seven (27) times* more discrete “target regions” than the number of exon 53 ASOs any POSA had—at that time—ever evaluated at a given time.

69. As Dr. Wood describes, as of June 2005 only two publications described testing morpholino ASOs for inducing dystrophin skipping: Gebiski (2003) and Aartsma-Rus (2004). Wood Suppl. Rpt. ¶¶ 39-43. These tests involved two different morpholino ASOs (one which was annealed to various DNA/RNA leashes), and neither targeted exon 53. Thus, a POSA reviewing the UWA Patents’ specification (which does not disclose any morpholino testing) with knowledge of the art would understand that, at that point, no POSA had reported testing more than a single “target region” with morpholino ASOs at a given time.

70. That 168 or 330 would be considered “large” numbers by POSAs is also shown by the literature. Popplewell (2010), for example, characterizes four prior studies as describing “*large screens* of AO bioactivity in vitro.” Popplewell (2010) at 108 (citing references “24,25,28,29”). Notably, this is five years after the UWA Patents’ priority date. These studies are:

[24] Popplewell LJ, Trollet C, Dickson G, Graham IR. Design of phosphorodiamidate morpholino oligomers (PMOs) for the induction of exon skipping of the human DMD gene. Mol Ther 2009;17:554-61.	Reports testing <i>sixty-six (66)</i> total morpholino ASOs across multiple exons. See Popplewell (2009) at 551).
[25] Wilton SD, Fall AM, Harding PL, McClorey G, Coleman C, Fletcher S. Antisense oligonucleotide-induced exon skipping across the human dystrophin gene transcript. Mol Ther 2007;15:1288-96.	Reports testing 470 total ASOs across multiple exons. See Wilton Mol Ther 2007.

<p>[28] Aartsma-Rus A, De Winter CL, Janson AAM, et al. Functional analysis of 114 exon-internal AONs for targeted DMD exon skipping: indication for steric hindrance of SR protein binding sites. <i>Oligonucleotides</i> 2005;15:284-97.</p>	<p>Reports testing <i>seventy-seven (77)</i> “new” ASOs across an “additional 21 exons” for a total of one-hundred fourteen (114) ASOs tested “thus far” across thirty-five (35) exons. <i>See</i> Aartsma-Rus (2005) at Abstract.</p>
<p>[29] Aartsma-Rus A, van Vliet L, Hirschi M, et al. Guidelines for antisense oligonucleotide design and insight into splice-modulating mechanisms. <i>Mol Ther</i> 2008;17:548-53.</p>	<p>Reports a “retrospective analysis of a series of <i>156 AONs</i>” across many exons. <i>See</i> Aartsma-Rus (2008) at Abstract.</p>

71. Three of the publications Popplewell (2010) characterizes as describing “large screens” analyze fewer ASOs than there are discrete “target regions” for the ’851 Patents’ claims. And two of them (Aartsma-Rus (2005) and Popplewell (2009)) report *less than half* as many.

72. Popplewell (2010) characterizes the Wilton Mol. Ther. (2007) publication as describing a “large screen.” That article explains that “[t]o date, we have evaluated 470 20MeAOs for targeted skipping of dystrophin exons 2-78, some of which have been described elsewhere.” Wilton Mol. Ther. (2007) at 6 (citing PCTAU200/000943—the priority application for the UWA Patents’, a 2006 publication and a 2007 publication). In other words, this publication is not reporting that the UWA researchers tested 470 ASOs in a single screen. Rather, it is reporting that, over their years and multiple sets of experiments testing ASOs targeted to the dystrophin gene, the UWA researchers tested a combined 470 2’O-Me ASOs (and a “limited” set of phosphorodiamidate morpholinos, for which the data is “not shown”). *See id.* at 6-7.

and the Wilton Mol. Ther. (2007) publication was “[r]eceived 24 August 2006” by the journal.

73. Therefore, in my opinion, the number of either 168 ('851 Patent) or 330 ('590 and '827 Patents) discrete exon 53 “target regions” would, as of June 2005, have been recognized by POSAs to be “vast.” These figures far exceed the number of exon 53 ASOs any group of POSAs had then-evaluated, and *no* morpholino exon 53 ASOs had then-been reported. And they likewise materially exceed the *total* number of ASOs directed at *any* dystrophin exon (114) that the University of Leiden researchers (a leading exon-skipping laboratory) had then-reported testing. *See* Aartsma-Rus (2005).

74. Importantly, however, the number of chemically-distinct ASOs meeting the structural claim limitations is not limited to the 168 or 330 distinct “target regions.” To calculate the *full scope* of the claims, one must also consider the chemical variabilities allowed by the claims when designing ASOs to these “target regions.” Because the claims allow for chemical variabilities, a POSA could design many chemically distinct ASOs that meet all structural claim limitations; and yet target the exact same “target region.”

75. To estimate the “full scope” of ASOs meeting the claims’ structural limitations—and not just the “target regions”—a POSA would first consider the chemical variabilities taught by the specification to be possible. As discussed above, *supra* Section VIII.B, the specification describes at least nine different options for modified bases and at least nineteen different options for chemical moieties. And Sarepta—in contending that VYONDYS 53 falls within the scope of the claims—has identified at least a twentieth chemical moiety option (5’ moiety including a TEG, as discussed above).

76. These options alone show that at least tens of thousands of ASOs meet all of the claims’ structural limitation. To conservatively simplify the calculations provided immediately below, I treated “base modifications” as a single variation (*e.g.*, as if only one of the nine options

for modifying bases were used with a given ASO at a time). The resulting estimate involves multiplying the number of discrete “target regions” by the possible number of “base modification” options and by the possible number of “moiety” options:

“Target Regions”		Base Modification		Moiety		ASOs
168	X	9	X	20	=	30,240
330	X	9	X	20	=	59,400

This simplified calculation provides a highly-conservative estimate of the number of ASOs that meet the claims’ structural limitations. As the specification states, “[i]t is not necessary f[o]r all positions in a given compound to be uniformly modified, and in fact *more than one* of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide.” ’851 Patent at 27:60-64 (emphasis added).

77. To apply this specification teaching when calculating an estimate, one must multiply together the number of modifications possible for each individual base (rather than just use the 9 options in my simplified calculation). An exemplary sequence of bases that meet the structural limitations of the claims is illustrated below: SEQ 195, where uracil bases are thymine bases:

SEQ ID No. 195
(uracils are thymines)

CTG AAG GTG TTC TTG TAC TTC ATC C

As shown, this 100% complementary sequence contains six (6) non-thymine/uracil **pyrimidines** (cytosine)¹⁰ and nine (9) purines (**adenine** and **guanine**) that each could be independently

¹⁰ Again, based on the limitation “wherein uracil bases are thymine bases,” I am conservatively assuming no modifications to thymines.

modified. From the specification disclosures noted above, there would be at least five options for modifying each cytosine ((1) *5-propynylcytosine*; (2) *5-methylcytosine*; (3) *other 5-substituted pyrimidines*; (4) *6-azapyrimidines* and (5) *no modifications*), five options for modifying each adenine ((1) *2-aminopropyladenine*; (2) *other N-2 substituted purines*; (3) *N-6 substituted purines*; (4) *0-6 substituted purines* and (5) *no modifications*) and four options for modifying each guanine ((1) *other N-2 substituted purines*; (2) *N-6 substituted purines*; (3) *0-6 substituted purines* and (4) *no modifications*).

78. The total possible number of unique combinations of base modifications for ASOs targeting a particular “target region” would be calculated through the following formula:

$$(\text{Options for each C})^{(\# \text{ of Cs})} \times (\text{Options for each A})^{(\# \text{ of As})} \times \text{Options for each G})^{(\# \text{ of Gs})}$$

For the particular “target region” above corresponding to SEQ ID No. 195 (+23+47), that calculation is $5^6_{(\text{cytosines})} \times 5^4_{(\text{adenines})} \times 4^5_{(\text{guanines})} = 1.0 \times 10^{10}$ (10 billion) unique combinations of base modifications. As a result, at least that many chemically distinct ASOs would be possible for this particular “target region,” even before accounting for chemical moieties or other chemical variations allowed by the claims. To identify the full scope of ASOs meeting the structural claim limitations across the claimed genus, this type of calculation would need to be performed individually for each of the 168 or 330 discrete “target regions.”

79. To determine the full scope, a POSA would also have to account for other possible chemical variations that are not explicitly mentioned by the specifications. Throughout this section, I have noted where the specification describes “classes” of chemical modifications or moieties that could be implemented. For example, the specification refers generically to using “a polyethylene glycol chain” as a chemical moiety, and golodirsén uses a particular type of polyethylene glycol chain: a *tri*-ethylene glycol (TEG). Except where specific subclasses or

examples are identified, my exemplary calculations have conservatively treated these “classes” as if they were a single option, and not a category of options.

80. Similarly, my calculations above did not account for possible variations in inter-nucleotide linkages. Using Sarepta’s exemplary general structure of an antisense morpholino subunit discussed above, *supra* Section VIII.B, for example, results in at least fifty-six (56) chemically distinct types of morpholino inter-nucleotide linkages: 4 (*Y1 options*) x 2 (*Z options*) x 7 (*X options*) = 56. Sarepta also allows for different intersubunit linkages to be used within a single antisense oligonucleotide. *Id.* at 283 (“In certain embodiments, the above intersubunit linkages, which are uncharged, may be interspersed with linkages that are positively charged at physiological pH, where the total number of positively charged linkages is between 2 and no more than half of the total number of linkages.”). This further increases the number of possible “morpholino” backbones an ASO could have, while still falling within the scope of the claims.

81. Thus, in my opinion a POSA would consider the claimed genus of each UWA Patent to be vast. Applying just the specification’s teachings regarding possible base modifications and chemical moieties, a POSA would conclude that the UWA Patents’ claimed structural requirements each encompass no fewer than tens of thousands of chemically distinct ASOs, and would estimate the total number as at least in the millions. Put differently, a POSA would recognize that the claims’ structural requirements encompass more than a *thousand times* as many ASOs as any group of POSAs had, in 2005, *ever* reported evaluating for exon 53 at a given time (Matsuo’s 12 exon 53 ASOs).

IX. THE UWA PATENTS LACK WRITTEN DESCRIPTION UNDER 35 U.S.C. § 112

82. The UWA Patents disclose only no testing of any species of antisense oligonucleotide that actually falls within the vast scope of the claimed genus. A person of ordinary

skill in the art would understand that the inventors did not have possession of the full scope of the claimed genus of antisense oligonucleotides with the claimed functionality of inducing exon 53 skipping. As such, it is my opinion that the asserted claims of the UWA Patents are invalid for failure to comply with the written description requirement.

A. The UWA Patents Do Not Disclose a Representative Number of Species That Cover the Scope of the Broad Genus of Antisense Oligonucleotides

83. As discussed above in Section VIII, the genus of claimed antisense oligonucleotides is extremely broad and structurally encompass no fewer than tens of thousands of antisense oligonucleotides, and likely at least millions.

84. Based on the Court's recent construction, the claims are limited to exon 53-skipping antisense oligonucleotides that are 100% complementary to a target region of dystrophin pre-mRNA. The specification discloses twelve different ASOs directed towards exon 53 and 100% complementary to dystrophin pre-mRNA, but a POSA would *not* consider them representative of the full scope of the claimed genus.

85. Only seven (7) ASOs—SEQ ID NO: 191 (25 bases long), SEQ ID NO: 192 (24 bases long), SEQ ID NO: 193 (31 bases long), SEQ ID NO: 194 (21 bases long), SEQ ID NO: 195 (25 bases long), SEQ ID NO: 196 (27 bases long), and SEQ ID NO: 198 (27 bases long)—of the twelve (12) 100% complementary exon 53-directed ASOs (SEQ ID Nos. 191 through 202), were reported to induce some level of exon 53 skipping. '851 Patent, tbl. 39. By contrast, SEQ ID NO: 199 (22 bases long), SEQ ID NO: 200 (25 bases long), SEQ ID NO: 201 (20 bases long), and SEQ ID NO: 202 (22 bases long)—despite being 100% complementary and designed specifically “based upon known or predicted motifs or regions [of pre-mRNA] involved in splicing”—did not induce any exon 53 skipping at all. *Id.* at 32:31-36. And SEQ ID No. 197 was “Not made yet.” Thus, the ASOs described in the UWA Patent specification are not representative of a genus of

100% complementary exon 53 skipping antisense oligonucleotides that are 20 to 31 bases in length at least because SEQ ID NO: 201 (20 bases long) and SEQ ID NO: 199 and 202 (22 bases long), did not induce exon 53 skipping and no 100% complementary 23, 26, 28 or 29 bases long ASO was ever tested for exon 53 skipping.

86. The specification of the UWA Patents discloses only a single antisense oligonucleotide that includes “at least 12 consecutive bases of SEQ ID NO: 195” and is 100% complementary to its target region. That ASO is SEQ ID NO: 195 itself (also referred to as “H53A(+23+47)”), and is exactly 25 bases in length. However, this sequence does not contain thymine bases, nor does it contain any morpholino chemistry. *See* ’851 Table 1A (“Description of 2’-O-methyl phosphorothioate antisense oligonucleotides” and listing SEQ ID NO: 195).¹¹ Dr. Wood uses the term “pseudo-species” to describes ASOs like these, and I may use that terminology from time-to-time. Wood Suppl. Rpt, n. 10. SEQ ID NO: 195 reportedly induced only “very faint exon skipping to 50 nM,” as shown below:

¹¹ Indeed,

see also Wilton Dep. Tr. at 177:11-179:10

TABLE 39

SEQ IDname	Antisense oligonucleotide	Sequence	Ability to induce skipping
191	H53A (+45+69)	CAU UCA ACU GUU GCC UCC GGU UCU G	Faint skipping at 50 nM
192	H53A (+39+62)	CUG UUG CCU CCG GUU CUG AAG GUG	Faint skipping at 50 nM
193	H53A (+39+69)	CAU UCA ACU GUU GCC UCC GGU UCU GAA GGU G	Strong skipping to 50 nM
194	H53D (+14-07)	UAC UAA CCU UGG UUU CUG UGA	Very faint skipping to 50 nM
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C	Very faint skipping to 50 nM
196	H53A (+150+176)	UGU AUA GGG ACC CUC CUU CCA UGA CUC	Very faint skipping to 50 nM

“Very faint skipping at 50 nM” does not meet the UWA Patent’s definition of “efficient antisense molecule.” *See* ’851 Patent at 33:13-16 (“Our definition of an efficient antisense molecule is one that induces strong and sustained exon skipping at transfection concentrations in the order of 300 nM or less.”). In my opinion, the sole disclosure of SEQ ID NO: 195 is insufficient to support the broad genus of antisense oligonucleotides encompassed by the claims of the UWA Patent.

87. Moreover, as explained by Dr. Wood, exon-skipping activity can vary greatly even among antisense oligonucleotides sharing a common nucleotide sequence. Wood Report ¶¶ 75-86; *see also* Wood Interference Declaration ¶¶ 68-77. Therefore, a POSA would not consider that the named inventors were in possession of a genus of antisense oligonucleotides having exon skipping activity merely because they shared 12 consecutive bases of SEQ ID NO: 195, particularly given the very low level of skipping demonstrated by SEQ ID NO: 195.

88. Indeed, there is no indication in the UWA Patents that comprising 12 consecutive bases of SEQ ID NO: 195 is even important for exon 53 skipping. The sole disclosure of “12 bases” in the specification of the UWA Patents is in a passage discussing exon 19 skipping. ’851 Patent at 23:58-24:3 (“With some targets such as exon 19, antisense oligonucleotides as short as

[REDACTED]

12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides.”) The UWA Patents make no mention of “12 bases” with respect to exon 53 skipping or SEQ ID NO: 195.

89. Further, the phrase “12 bases” is used in the above cited passage to describe overall antisense oligonucleotide length. It does not use the term “12 bases” to describe a “base sequence” component included within a longer antisense oligonucleotide with additional bases, as the “at least 12 consecutive bases” requirement operates to specify at least a portion of the “base sequence” recited in the UWA Patent claims. Indeed, [REDACTED]

[REDACTED]

[REDACTED]

Wilton Dep. Tr. at 228:1-229:17.

90. Furthermore, as explained by Dr. Wood, the deletion of as few as two nucleotides from an antisense oligonucleotide that induces exon skipping or changes to a significant number of nucleotides in an antisense oligonucleotide that induces exon skipping can reduce or eliminate such activity altogether. Wood Report ¶ 81; *see also* Wood Interference Declaration ¶ 74; Wilton, Steve D., and Susan Fletcher. “Antisense oligonucleotides in the treatment of Duchenne muscular dystrophy: where are we now?” *Neuromuscular Disorders* 15.6 (2005): 399-402 (“Displacing the annealing site of an AO by only a few bases can alter its exon skipping potential by more than an order of magnitude, hence AO targeting needs to be precise for efficient exon skipping.”); Wilton Dep. Tr. at 58:3-6. SEQ ID NO: 195 is comprised of solely 25 nucleotides overall. The claims, however, encompass antisense oligonucleotides that comprises 20 to 31 nucleotides, and, therefore, include antisense oligonucleotides that are shorter than 25 nucleotides.

91. In a 2007 publication, Dr. Wilton reported that “[t]he length of the AO has emerged as a major parameter, AOs of 25–31 nucleotides outperform shorter compounds.” Harding, P. L., et al. “The influence of antisense oligonucleotide length on dystrophin exon skipping.” *Molecular Therapy* 15.1 (2007): 157-166 (“Harding”) at 164; *id.* at Abstract (“AOs of 25–31 nucleotides are generally more effective at inducing exon skipping than shorter counterparts”). In that same publication, Dr. Wilton stated that “[o]verlapping 25mers were only able to induce very low levels of exon 53 skipping, whereas the 31mer induced consistent exon skipping at transfection concentrations as low as 10 nM.” *Id.* at 164; *see also* WILTON0017886 at 887 (“[REDACTED] Wilton Dep. Tr. at 218:1-220:14 ([REDACTED]); 80:13-81:9 ([REDACTED])”).¹²

92. In view of the unpredictability associated with changing the number of nucleotides in an antisense oligonucleotide, and in particular with shortening antisense oligonucleotides, a POSA would not conclude, based on the sole disclosure of SEQ ID NO: 195, that the named inventors were in possession of a genus of antisense oligonucleotides shorter or longer than SEQ ID NO: 195, that comprise at least 12 consecutive bases of SEQ ID NO: 195, that still exhibit exon 53 skipping.

93. The specification of the UWA Patents provide very limited data regarding what length would be considered optimal for exon 53 skipping antisense oligonucleotides. For example, of the exon 53 skipping antisense oligonucleotides disclosed in the UWA Patents, the only one

¹² [REDACTED]

[REDACTED] Wilton Dep. Tr. at 183:13-184:7; WILTON0017692 at 694.

that was reported to exhibit “strong skipping” was the 31 mer (+39+69). The 27 mers (+150+176 and +09-18) exhibited “very faint skipping at 50 nM” and “faint skipping at 600 nM,” respectively; the 25 mers (+45+69, +27+47 and -07+18) exhibited “faint skipping at 50 nM,” “very faint skipping at 50nM” and “no skipping” at all, respectively, while one 25 mer (+20-05) was “not made yet”; the 24 mer (+39+62) exhibited “faint skipping at 50 nM”; both 22 mers (-12+10 and +124+145) exhibited “no skipping” at all; the 21 mer (+14-07) exhibited “very faint skipping at 50 nM”; and the 20 mer (+07+26) exhibited “no skipping” at all. In my opinion, this limited and inconsistent data would not have informed a POSA that the named inventors had identified an optimal length for antisense oligonucleotides for exon 53 skipping. They certainly did not demonstrate that antisense oligonucleotides comprising 20 to 31 nucleotides in length were optimal.¹³ Moreover, the UWA Patents disclose a range of “20 to 31 bases” in the context of exon 19 skipping, not exon 53 skipping. *See* ’851 Patent at 23:58-24:3. The UWA Patents are silent as to how “20 to 31 bases” relates to exon 53 skipping.

94. Moreover, as stated in the specification, “[a]ttempts by the inventors to develop a rational approach in antisense molecule design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons.” ’851 Patent at 32:15-21 (further noting that “identification” had therefore required “empirical studies.”). Thus, by their own admission, the named inventors did not consider the rational design methodologies used for the specification’s Examples to be “reliable”:

These empirical studies involved the use of computer programs to identify motifs potentially involved in the splicing process. Other computer programs were also used to identify regions of the pre-

¹³ To the contrary, in a post-priority date publication reporting on some of the same exon 53 AOs as the UWA Patents, Dr. Wilton noted that AO length was an important factor for efficient exon 53 skipping based on results showing that a 31mer (SEQ ID NO: 193) was more effective than 25mers (SEQ ID Nos: 191 and 192) that overlapped in sequence. Harding at 164.

mRNA which may not have had extensive secondary structure and therefore potential sites for annealing of antisense molecules. Neither of these approaches proved completely reliable in designing antisense oligonucleotides for reliable and efficient induction of exon skipping.

Id. at 32:22-36.

95. ASOs that are entirely 100% complementary to the dystrophin pre-mRNA are no exception to the high levels of unpredictability in the art of exon skipping. The UWA researchers' own work illustrates this point. As discussed above, the specification reports that the UWA inventors did not choose the annealing sites for their ASOs at random—their “empirical studies involved the use of computer programs to identify motifs potentially involved in the splicing process” or “identify regions of the pre-mRNA which may not have had extensive secondary structure and therefore potential sites for annealing of antisense molecules.” ’851 Patent at 32:22-27. “Annealing sites on the human dystrophin pre-mRNA were selected for examination, initially based upon known or predicted motifs or regions involved in splicing” and “2OMe antisense oligonucleotides *were designed to be complementary to the target sequences under investigation.*” *Id.* at 32:31-36 (emphasis added).

96. In other words, the UWA researchers used an intelligent design approach to select 100% complementary ASOs that would bind to particular target regions of pre-mRNA that they expected would produce exon skipping activity. One might attempt to target particular “motifs” or “regions” involved in splicing with the idea that the ASO’s binding would interfere with cellular splicing machinery, and thus induce skipping. As Dr. Wood explains, one might attempt to target particular regions without “extensive secondary structure” because ASOs have to three-dimensionally “fit” into the pre-mRNA’s three-dimensional structure if they are to effectively pair with the pre-mRNA. Wood Suppl. Rpt. ¶¶ 11-21. But analyzing RNA structure is a field that

remains in its infancy, and I agree with Dr. Wood that this adds considerable unpredictability to the field. *See id.* As the UWA researchers themselves reported, “[n]either of these approaches proved completely reliable in designing antisense oligonucleotides for reliable and efficient induction of exon skipping.” ’851 Patent at 32:27-30.

97. The UWA researchers elaborated on this further in their Wilton Mol. Ther. (2007) article discussing that by that point, of the 470 2’O-Me ASOs they had tested across dystrophin exons 2 through 78, only “two-thirds were found to induce detectable exon skipping.” Wilton Mol. Ther. (2007) at 1293. And they reaffirmed that “induced exon skipping was *not as simple as targeting the obvious donor or acceptor splice sites.*” *Id.* (emphasis added). “The inability of some splice sites or motifs to respond consistently to AO intervention suggests that other factors, include binding of protein factors, pre-mRNA, or AO secondary structure, may prevent the AOs from annealing and redirecting splicing.” *Id.*

98. These reports of unpredictability with activity induced by 100% complementary ASOs designed by the UWA researchers is consistent with the data the specification reports for exon 53. Four of the eleven exon 53-targeted ASOs tested (36%) achieved “No skipping,” including one that shared three bases with SEQ ID NO: 195 (SEQ ID NO: 202 (H53A(+07+26))). ’851 Patent at tbl. 39. The most active exon 53 ASO was 31-mer SEQ ID NO: 193 H53A(+39+69), which was reported to induce “Strong skipping to 50 nM.” *Id.* But two shortened versions that shared *all* of their bases with that ASO—25mer SEQ ID NO: 191 (H53A(+45+69) and 24mer SEQ ID NO: 192 (H53A(+39+62))—were reported to have starkly lower activity: “Faint skipping at 50 nM.” *Id.* SEQ ID NO: 195 (H53A(+23+47))—despite sharing 9 bases with SEQ ID NO: 192 and SEQ ID NO: 193 and 3 bases with SEQ ID NO: 191—was reported to induce even less skipping: “very faint skipping to 50 nM.” *Id.* Again, all of these

ASOs were 100% complementary to dystrophin pre-mRNA and many shared common bases with each other, and yet significant decreases in exon-skipping activity (including to “No skipping”) were observed as the annealing site and length changed.

99. Importantly, much of the evidence of unpredictability discussed by Dr. Wood—and relied upon by Sarepta and UWA during prosecution of the ’851 Patent and the ’007 Interference—relates to 100% complementary ASOs. During prosecution of the ’851 Patent, the examiner issued a Non-Final Rejection on Oct. 5, 2017 in which the pending claims—which are identical to those that ultimately issued—were rejected, among other things, as obvious over van Ommen (WO2004/083432) and Koenig et al. ’851 Patent Prosecution History, Non-Final Rej. (Oct. 5, 2017) at 2-5; Claims (Sep. 15, 2017). In that rejection, the examiner argued that “one of skill in the art would have been motivated to use the sequence of [van Ommen’s] h53AON1 to arrive at oligonucleotides of 20 nucleotides and having 12 nucleotides of SEQ ID No. 195 (which overlaps with 3 nucleotides of h53AON1).” ’851 Patent Prosecution History, Non-Final Rej. (Oct. 5, 2017) at 4. h53AON1 is an 18mer ASO that is 100% complementary to positions h53A(+45+62) of exon 53’s pre-mRNA.

100. In response to this rejection, Sarepta and UWA spent pages arguing that “there was a significant level of unpredictability associated with selecting specific antisense oligonucleotide sequences to induce effective dystrophin exon skipping.” ’851 Patent Prosecution History, UWA Args./Remarks (Jan. 5, 2018) at 10-17. They first cited the UWA specification as evidence of unpredictability, characterizing its teachings as follows:

- “[T]he specification as originally filed notes that the size or length of an antisense oligonucleotide is not predictive of its efficacy”;
- “Applicants have found that there is no standard motif that can be blocked or masked by antisense molecules to redirect splicing.”

Id. at 10.¹⁴ As discussed above, I agree with these characterizations that the art remained highly unpredictable even after the UWA researchers' work with 100% complementary ASOs.

101. Sarepta and UWA then proceeded to cite a series of exon-skipping studies performed through the June 2005 priority date that, according to Sarepta and UWA, demonstrated that “[a]s each antisense oligonucleotide must be empirically analyzed, the results are *unpredictable*,” that “*significant experimentation* is required to arrive at specific oligonucleotides,” and that there is an “unpredictable impact that length and nucleotide composition make with respect to efficiency in inducing exon skipping.” *Id.* at 10-13 (emphases theirs). Notably, from these studies, Sarepta and UWA repeatedly highlighted instances where differing results were obtained from two or more 100% complementary ASOs that had shared bases. *See id.* at 11 (discussing van Ommen’s ’952 Publication and characterizing Aatsma-Rus (2002) as teaching “that there is no correlation between the length or sequence of the oligonucleotide and its effectiveness at inducing exon skipping”); *id.* at 11-12 (highlighting disparate results achieved by certain UWA researchers with “overlapping” 100% complementary ASOs in Mann (2002)); *id.* at 12-13 (highlighting disparate results achieved by Leiden researchers with overlapping 100% complementary ASOs in Aartsma-Rus (2005)). From these studies, they argued to the Patent Office that it was “a hit-or-miss proposition in terms of whether any given antisense oligonucleotide will be capable of inducing skipping, *even in situations where the*

¹⁴ Similarly, in the ’007 Interference, Sarepta and UWA characterized the UWA patents’ specification as illustrating the need to test every individual exon 53-skipping ASO. UWA Reply 1 at 9 (“[T]he need to test each and every AON is also reflected in UWA’s specification, which states that ‘[o]nce efficient exon skipping [has] been induced with one antisense molecule, subsequent overlapping antisense molecules may be synthesized and then evaluated.’”).

antisense oligonucleotides are very similar to each other in terms of nucleotide sequence, and other variables concerning the chemical backbone are fixed.” Id. at 13 (emphasis theirs).

102. Sarepta and UWA then went farther, telling the patent office that “[t]he recognition of the lack of predictability in the field of exon skipping continued beyond 2005,” *i.e.*, continued even *after* the UWA Patents’ June 2005 priority date. ’851 Patent Prosecution History, UWA Args./Remarks (Jan. 5, 2018) at 13. Again, they cited publications by others demonstrating continued unpredictability, including highlighting a 2007 statement that “several years after the first attempts at dystrophin exon skipping with AOs [antisense oligonucleotides], *there are still no clear rules to guide investigators in their design*,” and a 2009 statement that “in general *a trial and error procedure* is still involved to identify potent AONs.” *Id.* at 13-14 (emphasis theirs). They also cited a Wu (2011) article as yet further evidence that, in their words, “selecting specific antisense oligonucleotide sequences to induce effective dystrophin exon skipping remains [*i.e.*, as of January 2018] an unpredictable exercise.” *Id.* at 14-15.

103. Notably, what Sarepta and UWA again highlighted from Wu (2011) were instances where “overlap[ping]” 100% complementary ASOs exhibited starkly different activity:

For example, the 19- and 20-mer AOs hE50 AO2PS and hE50AO3PS were inactive. Increasing the length to 22 and 27 bases (hE50 AO4PS and hE50 AOSPS, respectively) resulted in increased activity, but a further increase to 32 bases (hE50 AO6PS) decreased activity significantly.

Id. at 15 (concluding that “Wu et al. demonstrate that increasing or decreasing AO length results in unpredictable effects on exon skipping.”).

104. Sarepta and UWA made largely the same arguments in the ’007 Interference which, like the ’851 Patent, related to exon 53-skipping ASOs.¹⁵ In one of its motions, Sarepta and UWA

¹⁵ Specifically, the ’007 Interference related to the UWA Patent 8,455,636 (which claims priority to the same June 2005 patent application as the ’851 Patent, and I understand to have substantially

began by characterizing the art around the time of the filing dates of the “competing applications” (i.e., 2003-2005) as follows:

When the competing applications in this interference were filed, a handful of specific operative exon skipping antisense oligonucleotides (“AONs”) targeting exon 53 had been discovered, and the path to identifying others was largely unknown. Both parties submitted broad generic claims in the hope that identification of broader families of operative AONs would follow predictably from those narrower discoveries. **Subsequent experience has revealed that operative sequences are actually highly unpredictable, varying with parameters such as nucleobase sequence, length, backbone chemistry, and internucleotide linkages.**

UWA Mot. 1 (Nov. 18, 2014) at 1 (emphasis added). They elaborated further:

It is now known that **many factors influence the binding of an AON to its target, including AON length, target accessibility, nucleobase sequence, modifications to the chemical backbone, Watson-Crick “mismatches,” and modifications to the internucleotide linkages.** Consequently, there is **tremendous variability and unpredictability in the efficacy of different AONs targeted to different regions of the dystrophin pre-mRNA, and each different AON needs to be empirically tested.**

Id. at 4 (emphasis added). Notably, only one of these listed factors is “Watson-Crick ‘mismatches’”—even with the UWA Patents’ claims limited to 100% complementary ASOs, there is still unpredictability caused by variations in at least ASO length, target accessibility (secondary structure), nucleobase sequence, and internucleotide linkages.

105. As evidence supporting this characterization of the unpredictability of exon 53 skipping, Sarepta and UWA cited much of the same evidence as in the ’851 Patent’s prosecution. *See id.* at 4-5; *see also id.* at 17 (“Exon skipping of dystrophin pre-mRNA was a nascent and highly unpredictable technology as of the time of the invention (***and remains so today***), as demonstrated by”) (emphasis added). For example, they again highlighted how Wu (2011) disclosed

the same specification) and Academisch Ziekenhuis Leiden’s (“AZL”) Application No. 11/233,495, which was published as U.S. 2006/0147952 A1, filed on Sep. 21, 2005, and claims priority to PCT/NL03/00214 (filed Mar. 21, 2003).

multiple 100% complementary ASOs “having overlapping nucleobase sequences” and yet differing activity. *Id.* at 5 (“The 20-mer AO3PS induced no detectable exon skipping. The 22-mer AO4PS, differing only in having two additional nucleotides complementary to the *DMD* gene, induced detectable exon skipping, but only in 4% of cells. Adding an additional five nucleotides increased exon skipping to 21%. However, adding five more nucleotides largely abrogated this effect.”). They likewise relied upon opinions from Dr. Wood that he (still) offers here. *See, e.g., id.* at 7 (“The unpredictability of exon skipping occurs in part because of a ‘Goldilocks’ dilemma. If the AON does not bind tightly enough, exon skipping will not occur. But exon skipping also will not occur if the AON binds too tightly, because AON turnover from transcript to transcript is essential. (Exh. 2081 [Wood Interference Decl.] at ¶¶ 50, 74-75; Exh. 2014 at 552; Exh. 2020 at 259.) Moreover, because the AON needs to access conformationally complex “target” sequences, changes in length have unpredictable effects. (Exh. 2081 [Wood Interference Decl.] at ¶¶ 69-70.)”).

106. The “Goldilocks” dilemma is equally applicable to the genus of ASOs encompassed by claims of the UWA Patents. Even with 100% complementarity, the binding affinity and secondary structure of claimed ASOs will vary based on parameters such as length, “target region,” use of modified bases and chemical moieties. The specification, for example, expressly teaches that modified bases may be used to alter “the binding affinity of the oligomeric compounds of the invention.” ’851 Patent at 27:37-39; *see also id.* at 27:42-46 (noting also that “5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications”). As such, this presents another reason why the 100% complementary exon 53 ASOs disclosed in the specification are not representative of the claimed

genus. As discussed above, none of the UWA Patents' disclosed ASOs were tested with a chemical moiety, nor with any base modification, nor with even a morpholino backbone. And yet, the claims encompass ASOs that have such features.

107. Ultimately, Sarepta and UWA persuaded the Patent Office to accept their characterization of the exon 53-skipping art in both the '007 Interference and the '851 Patent's prosecution. In their January 5, 2018 response to the examiner's Non-Final Rejection, Sarepta and UWA highlighted that "Interference No. 106,007 ('the '007 interference') concern[ed] exon 53 antisense oligonucleotides for DMD," that "the PTAB considered the foregoing evidence as representative of the state of the art with Exhibits 2010 and 2015 in Interference 106,007 corresponding to Aartsma-Rus and Wu et al." and that "Unpredictability in this art was determined by the PTAB to have existed at the time of the instant invention (and years afterwards)." Args./Remarks (Jan. 5, 2018) at 15-17. In the examiner's following Final Rejection, the obviousness rejection was withdrawn in response to Sarepta' and UWA's arguments. Final Rej. (Apr. 4, 2018).

108. Although certain publications cited by Sarepta and UWA during prosecution and the '007 Interference discuss ASOs directed towards exons other than exon 53, I agree with Sarepta and UWA that they illustrate the unpredictability of even 100% complementary exon 53-skipping ASOs. First, as discussed above, the exon 53 work reported in the specification exhibits the same type of unpredictable changes in activity (even with 100% complementary ASOs targeted to overlapping target regions) as are detailed in the cited publications for other exons. And second, as also discussed above, the specification itself characterizes the field as unpredictable.

109. Additionally, by requiring that the claimed antisense oligonucleotide "induces exon 53 skipping," the UWA Patent claims encompass antisense oligonucleotides exhibiting any level

[REDACTED]

of exon 53 skipping, including antisense oligonucleotides exhibiting a level of exon skipping that has a therapeutic effect. There is no disclosure in the UWA Patents however, that shows the inventors were in possession of a genus of antisense oligonucleotides having a therapeutic effect. Indeed, SEQ ID NO: 195 reportedly only exhibited very faint skipping in an in vitro assay.

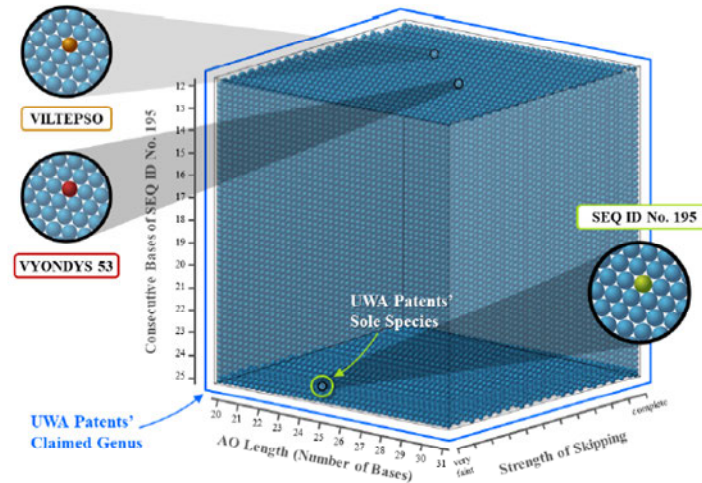
110. That the specification discloses a different 100% complementary ASO (SEQ ID NO: 193 (H53A(+39+69) reported to exhibit “Strong skipping to 50 nM” does not mean that it discloses “species” representative of the claims’ functional breadth. Like all disclosed 100% complementary ASOs other than SEQ NO: 195 itself, SEQ ID NO: 193 does not fall within any claim of the UWA Patents because it does not meet multiple claim elements: it was not evaluated as a morpholino, it does not have thymine bases, and it does not have “at least twelve consecutive bases of SEQ ID NO: 195.” There is no disclosure of any ASO in the specification that meets the sequence limitations of the claims and has *more* than “Very faint skipping,” much less any such ASO that has—like the later-discovered golodirsen and viltolarsen ASOs—sufficiently high exon skipping so as to be therapeutically effective at increasing dystrophin production in patients. That a 100% complementary ASO *outside* the claimed genus ostensibly exhibited “Strong skipping” does not mean that a POSA would understand the inventors to possess therapeutic ASOs *within* the genus. In fact, given that the specification only qualitatively describes the skipping induced by SEQ ID NO: 193 as “strong,” in my opinion no POSA could reliably conclude from the scant information disclosed by the specification that SEQ ID NO: 193 would have therapeutic levels of activity.¹⁶

¹⁶ As described below, [REDACTED]

111. In my opinion, the established unpredictability in the art (even with 100% complementarity ASOs) illustrates why a POSA would not consider the sole pseudo-species disclosed by the specification to be representative of the full scope of the claimed genus. Because the specification discloses only a single 25mer pseudo-species, a POSA has no basis to attempt to extrapolate expected activity of ASOs across the untested lengths and target regions claimed (particularly shorter 20 to 24-base ASOs), much less do so for ASOs having an entirely untested chemistry (morpholino). That the pseudo-species reportedly induced only “very faint” skipping only adds to the ambiguity. No POSA would consider a single, weak-skipping 2’OMe ASO predictive of a broad range of morpholino ASOs ostensibly having activity too. The art had repeatedly shown (as Sarepta and UWA argued to the Patent Office) that even minor chemical modifications could (and often did) completely eradicate observed exon-skipping activity.

112. As examples, Sarepta asserts that the genus encompasses at least VYONDYS 53 (golodirsen – a 25mer) and VILTEPSO (viltolarsen – a 21mer), but the specification discloses nothing similar to these ASOs. Considering that it has uracil bases, the SEQ ID No: 195 ASO tested by the UWA inventors shares only *eight (8)* of its 25 bases (32%) bases with VYONDYS 53 and VILTEPSO. There is no data whatsoever from which a POSA could conclude that the “very faint” activity observed with SEQ ID No: 195 would be preserved when changing the chemical backbone, shortening the ASO by four bases, and moving it to a substantially different target region that overlaps with less than half of SEQ ID No: 195. Indeed, VILTEPSO ultimately achieved starkly different (*therapeutic*) levels of exon skipping activity, despite its dissimilarity

to SEQ ID No: 195. I may rely upon visuals, such as the one below, to illustrate SEQ ID No: 195's dissimilarity along the claimed parameters from later-discovered species.



113. Thus, in view of the vast number of antisense oligonucleotides encompassed by the UWA Patent claims, the unpredictability in the field of exon skipping, and the named inventors' own admission in the specification of the UWA Patents, as well as in subsequent publications and at deposition, it is my opinion that the sole disclosure of SEQ ID NO: 195 is insufficient to be representative of the genus of antisense oligonucleotides that falls within the scope of the claims of the UWA Patents. Accordingly, in my opinion, the claims of the UWA Patents are invalid for lacking written description support.

B. The UWA Patents Do Not Disclose a Structural Feature Common to the Members of the Claimed Genus

114. The UWA Patents also do not disclose a structural feature common to the members of the claimed genus. I understand that one way to show that the inventors of UWA Patents were in possession of the claims, a POSA reading their common specification must be able to envision a structural feature of the claimed morpholino antisense oligonucleotides critical for inducing exon 53 skipping.

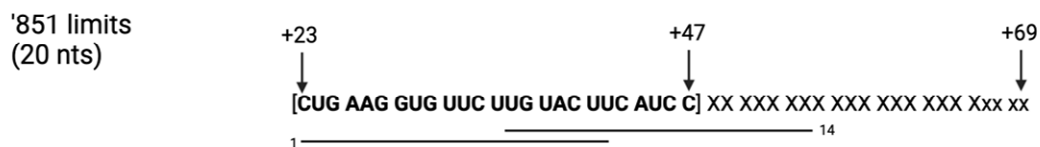
115. As discussed above, *supra* Section VIII, even taking into account the Court's updated construction of "base sequence" requiring 100% complementarity and all other structural requirements of the claims, the claimed genus of each UWA Patent still permits great structural and functional variability. Put differently, although the claims require each member of the genus to meet the same set of structural criteria, the *way* in which each member actually meets that structural criteria may differ.

116. The claimed morpholino antisense oligonucleotides of the UWA Patents are each 20 to 31 bases in length and include a base sequence comprising at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), wherein uracil bases are thymine bases, and 100% complementary to a target region. For the '851 Patent, that "target region" must also be within h53A(+23+69). The claimed antisense oligonucleotides are further required to induce exon 53 skipping. However, the UWA Patents only disclose a single antisense oligonucleotide sequence, SEQ ID NO: 195 (25 nucleotides long) that induces "very faint" exon 53 skipping activity according to Table 39 (and, as mentioned above, that antisense oligonucleotide in Table 39 is a 2'-O-Me with uracil bases, and not a morpholino having thymine bases, as required by the UWA Patent claims).

117. A POSA cannot envision what structural features are common to all functional species of the UWA Patent claims based on this single disclosure of SEQ ID NO: 195. As discussed by Dr. Wood and above, even near identity of nucleotide sequence is not predictive of exon skipping capability; indeed, the UWA Patents themselves show that a change in only a single nucleotide can be dispositive of exon skipping activity. Wood Report ¶ 193 (H16A(-06+19) induced skipping at 25nM, H16A(-07+19) did not); *see also id.* ¶ 81 (deletion of as few as two nucleotides from an antisense oligonucleotide that induces exon skipping can reduce or eliminate

ID NO: 195, and wherein uracil bases are thymine bases. Neither antisense oligonucleotide's base sequence shares any overlapping bases: ASO 32 includes (+36+65) of SEQ ID NO: 195, whereas ASO 1 includes (+5+34) of SEQ ID NO: 195. *See also supra* Section VIII.A (showing that the farthest 5' and farthest 3' target regions for the '590 and '827 Patents have no overlap). As the '590 and '827 Patents' claims do not even require every member to share any common sequence of bases, one of ordinary skill in the art cannot envision, based on sequence alone, which members of the claimed genus share a common structural feature that is associated with exon 53 skipping activity.

121. For the '851 Patent, the additional limitation that the "target region" must be within h53A(+23+69) does, as a practical matter, make it so that there is some overlap in each ASO's target region. *See supra* Section VIII.A (showing the farthest 5' and farthest 3' target regions). As shown, however, the shared overlap in "target region" across is only seven bases (the farthest 5' 20mer "target region" compared to the farthest 3' "target region" for any length). For example, the farthest 5' 20mer "target region" spans h53A(+23+42) and the farthest 3' 31mer "target region" spans h53A(+36+66).



122. Put differently, the **only** region of dystrophin pre-mRNA that all species of the '851 Patent commonly target are the seven bases spanning h53A(+36+42). Thus, a POSA would recognize that, as a consequence of the claims' requirements, the only "structure" commonly shared across each and every species within the '851 Patent's claimed genus is being a morpholino

having 100% complementarity to dystrophin pre-mRNA with seven bases complementary to h53A(+36+42) and thymine bases.

123. In my opinion, a POSA would understand that the specification nowhere discloses any correlation between complementarity to these seven bases and reliably or predictably achieving the claimed function: inducing exon 53 skipping. When the specification reports its results for exon 53, it only qualitatively reports what level of skipping each 2'O-Me ASO (with uracil bases) and a "cocktail" test ostensibly achieved. *See* '851 Patent at col. 64-65. Apart from noting—as is evident from Table 39—that "H53A(+39+69) [SEQ ID NO:193] induced the strongest exon 53 skipping," the specification makes no effort to interpret the reported exon 53-skipping results. *Id.* The specification does not include any discussion or explanation regarding why the inventors believed particular exon 53 ASOs exhibited different levels of skipping or what structures the inventors believed might be important for achieving exon skipping in exon 53 specifically. It makes no mention whatsoever of the seven bases spanning h53A(+36+42), much less suggest that targeting those seven bases in a 100% complementary morpholino ASO with thymine bases would not only reliably and predictably induce exon 53 skipping, but do so across the variable lengths claimed, and across the various chemical variations disclosed in the specification allowed by the claims (*e.g.*, nucleobase modifications and substitutions and chemical moieties).

124. If anything, the specification and state of the art would affirmatively convey to a POSA that the shared structure is **not** sufficient to reliably and predictably induce exon 53 skipping across the full scope of the genus. Because the UWA researchers only report testing 2'O-Me ASOs with uracil (and other natural) bases, there is no disclosure (much less data) in the specification that would inform a POSA how (1) altering the chemistry to morpholinos with thymine bases; (2)

implementing the base modifications taught by the specification; and/or (3) implementing the chemical moieties taught by the specification would affect an ASO's activity. Indeed, the specification contains no examples of the named inventors testing ASOs with such chemical features for any exon.

125. Further, the shared seven-base overlap h53A(+36+42) amounts to between 22.5% (31mer) and 35% (20mer) of the claimed ASOs. But, as discussed above—and detailed by Sarepta and UWA in the '851 Patent's prosecution and the '007 Interference—POSAs would be well-aware of the unpredictability in the art, and that ASOs with far more overlapping targets have exhibited disparate functional results. And there is no data from the specification from which a POSA could conclude that those seven bases correlated with inducing exon 53 skipping—the *only* ASO disclosed with a target region that encompasses h53A(+36+42) is SEQ ID NO: 195 itself, which exhibited only “Very faint skipping to 50 nM.” All other active ASOs (including every ASO reported as being more active) did not—like each and every claimed ASO—target bases h53A(+36+42).

126. Thus, in my opinion the specification fails to disclose a common structural feature of the claimed morpholino antisense oligonucleotides that is correlated with the claimed function of inducing exon 53 skipping.

C. The UWA Patents Do Not Define A “Hot-Spot” Amenable to Exon-Skipping Spanning Positions +23 to +69 of Exon 53

127. I understand that Sarepta has asserted that the UWA Patents identified “a ‘hot-spot’ amenable to exon-skipping ... within exon 53 spanning from positions 23 to 69.” Sarepta Final Validity Contentions at 8. I disagree that the UWA Patents define such a region. To the contrary, given that SEQ ID NO: 195 was characterized as inducing only “very faint skipping,” a POSA would not view the region, including positions +23 to +47, as a “hotspot.”

[REDACTED]

128. Post-priority date evidence supports that Dr. Wilton and his co-inventors neither recognized nor appreciated the +23 to +69 region of exon 53 to be a “hotspot.” First, in a 2007 publication, Dr. Wilton characterized the “hotspot” as “39–69 bases within the 212 base long exon 53.” Harding at 164; *see also* Wilton, Steve D., et al. “Antisense oligonucleotide-induced exon skipping across the human dystrophin gene transcript.” *Molecular Therapy* 15.7 (2007): 1288-1296 (testing 470 2’-O-Me antisense oligonucleotides across exons 2-78, but only exon 53 sequence discussed was +39+69); WILTON0026361. Consistent with these published accounts, deposition testimony, [REDACTED] all indicate that his exon 53 optimization efforts were focused on the +39 to +69 region, not +23 to +69.

129. Abbie Adams (née Fall) was the member of the Wilton laboratory [REDACTED]
[REDACTED] Wilton Dep. Tr. at 139:8-13. Ms. Adams [REDACTED]
[REDACTED] Adams Dep. Tr. at 72:8-18; WILTON0012314; Wilton Dep. Tr. at 111:4-13. [REDACTED]
[REDACTED]
[REDACTED]. Adams Dep. Tr. at 80:15-22, 81:3-10; SRPT-VYDS-0162960 at 055-097. In my opinion, this work shows that the named inventors did not view the “hot-spot” for exon 53 to include sequence upstream (5’) of +39 at this time.

130. A [REDACTED]
[REDACTED]
[REDACTED].¹⁷

¹⁷ The published Popplewell manuscript lists Dr. Wilton as a co-author and describes “previous studies by the Leiden group” as “suggest[ing] that the optimal 2’OMePS AO is targeted to the sequence +46+63 of exon 53.” Popplewell 2010 at 108-109. Popplewell characterized the known

[REDACTED], [REDACTED]

131. [REDACTED]

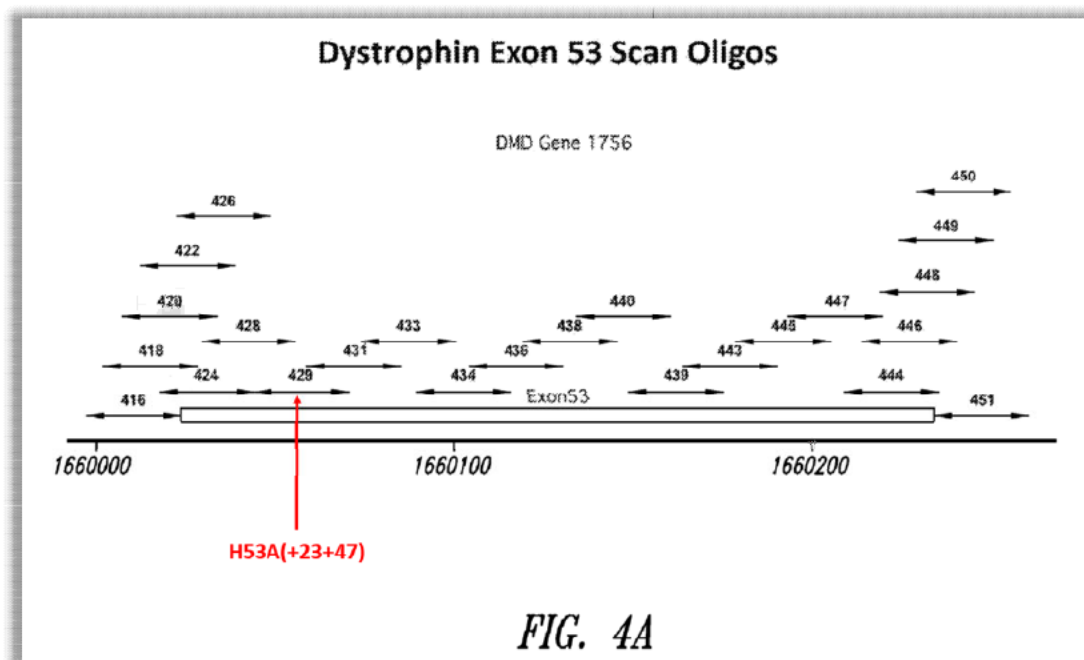
. In my opinion, this shows a lack of recognition or appreciation by the

transcript. Mol Ther 2007; 15:1288-96).

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inventors that the “hot-spot” extended to +23 at any time, much less before the priority date of the UWA Patents, as Dr. Wilton and Sarepta now claim. Wilton Dep. Tr. at 108:15-19 ([REDACTED]).

132. Later work by Sarepta shows that they too did not recognize that the inventors of the UWA Patents had identified a “hotspot” skipping region spanning positions +23+69 of exon 53. In U.S. Patent Publication No. 2010/0130591 A1 (“’591 Publication”), inventors Sazani and Kole made and tested a “series of [24] overlapping antisense PPMOs that target human dystrophin exon 53 ... each 25 bases in length.” ’591 Publication ¶ [0293]. Rather than focusing on the alleged +23+69 “hotspot” region Sarepta now alleges is described in the UWA Patents, Sazani and Kole’s PPMO’s targeted the entirety of exon 53:



’591 Publication at Figure 4A. And they designed this array of PPMOs despite being fully aware of Dr. Wilton’s work in the UWA Patents. *Id.* ¶ [0293] (“SEQ ID NO: 429 proved identical to H53A(+23+47) which is listed as SEQ ID NO: 195 in WO2006/000057.”).

133. But even if Sarepta is correct—they are not—that a POSA would understand the UWA Patents to define a “hotspot” region spanning from positions +23 to +69 in exon 53, that understanding still would not be sufficient to provide written description support for the entire genus of antisense oligonucleotides falling within that range. I understand that a written description must provide more than a mere wish or hope that the invention would work. As Dr. Wilton himself admitted, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]; *see also* Meloni Dep. Tr. at 22:12-23:3, 28:20-12.¹⁹

¹⁹ I understand that Sarepta has argued that “[n]umerous antisense oligonucleotides that share the characteristics of H53A(+23+47) and are within the scope of the claims of the Wilton Patents have been tested and shown to induce skipping of exon 53.” Sarepta Final Validity Contentions at 29. But none of these “[n]umerous antisense oligonucleotides” are disclosed in the UWA Patents, and therefore, are irrelevant to the question of whether the disclosure of the UWA Patents conveys to those skilled in the art that the inventors were in possession of the claimed invention. Indeed, as UWA itself argued in the UWA Interference, such extrinsic evidence of species falling within the scope of claims “is irrelevant for written description purposes, as it comes too late to demonstrate possession of the invention as of the filing date.” UWA Reply 1 (to AZL Opposition 1) at 11.

[REDACTED]

D. [REDACTED]

134. Although I understand that written description is judged from the disclosure within the four corners of the patent disclosure, I nevertheless looked at [REDACTED] to see whether they shed light on the interpretation or significance of the data in the UWA Patents.

[REDACTED]

135. [REDACTED]

[REDACTED]

136. [REDACTED]

[REDACTED]

137. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

138. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

20 [REDACTED]

[REDACTED]

[REDACTED]

SRPT-VYDS-0156085 at 6172.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

2. Once the problem is identified, the next step is to define the objectives and goals of the project. This helps to clarify what needs to be achieved and provides a clear direction for the team.

3. The third step is to develop a plan or strategy to address the problem. This involves breaking down the problem into smaller, manageable tasks and determining the resources needed to complete each task.

4. The fourth step is to implement the plan. This involves assigning tasks to team members, setting deadlines, and monitoring progress to ensure that the project is on track.

5. The final step is to evaluate the results of the project. This involves comparing the actual outcomes against the objectives and goals to determine the effectiveness of the project and identify areas for improvement.

21

[REDACTED]

139. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

SRPT-VYDS-0156085 at 6273 ([REDACTED])

[REDACTED]); *see also* SRPT-VYDS-0156085 at 6089 (“[REDACTED]”). [REDACTED]

[REDACTED]

140. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]



SRPT-VYDS-0158109 at 8145; Adams Dep. Tr. at 68:20-69:14 (

[REDACTED]

141. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

142. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

143. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

144. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

145. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

²² [REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Indeed,

in the Harding publication discussed above, H53A(+39+62) and H53A(+45+69) did not induce any discernible skipping at 50 nM (or even 100 nM), *contrary to what was reported in Table 39 of the UWA Patents*. See Fig. 4b from Harding (reproduced below); *see also* WILTON0012314

[REDACTED]

[REDACTED]

[REDACTED]

23

[REDACTED]

24

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

146. In sum, [REDACTED]

[REDACTED]

[REDACTED]

147. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

148. Further, Dr. Wilton has studied several other exons, but I was unable to locate even a single instance in any patent, publication, [REDACTED] in which he referred to a region of another exon as a “hot spot.”

149. For example, in addition to the 12 ASOs targeting exon 53, the '851 Patent discloses ASOs targeting 36 other exons. SRPT-VYDS-0002641 at 688-694. In 2011, Dr. Wilton filed another international patent application (“the '350 Application”) disclosing more than 275 ASOs targeting 29 different exons. SRPT-VYDS-0228438. Data included for other exons in both



the '851 Patent and the '350 Application further demonstrate why it is not possible to determine whether a “hot spot” exists based on the data for only a few ASOs.

ASO	Ability to Induce Exon Skipping
H3A(-06+20)	No skipping
H3A(+30+50)	Moderate 20-600 nM
H3A(+30+54)	Moderate 100-600 nM
H3A(+30+60)	Moderate skipping to 20 to 600 nM
H3A(+35+65)	Working to 300 nM
H3A(+37+61)	No skipping
H3D(+46-21)	No skipping
H3D(+19-03)	No skipping

SRPT-VYDS-0002641 at 703. Based on Dr. Wilton’s description of a hot spot this data supports the identification of a hot spot between +30 and +65 of Exon 3. *See* Wilton Tr. 224:16-18 (“In defining the amenable region plus 23, plus 48, that started the exon skipping and I continued down to 69.”); *id.* at 22:4-13. This region could have been defined as the region between annealing site H3A(+30+50) (“moderate skipping to 200 to 600 nM”) and H3A(+35+65) (“work[s] to 300 nM”). Two additional ASOs also induce exon skipping within this region: H3A(+35+65) and H3A(+30+54). *Id.*

150. The data reported for Exon 3 is similar to the “hot spot” Dr. Wilton purports to have described in the '851 patent as being defined by the H53A(+23+47) and H53A(+39+69) ASOs with two ASOs, H53A(+39+62) and H53A(+45+69) binding within that region which also induce exon skipping. *See id.* at 717. Yet, the data reported for Exon 3 also includes H3A(+37+61), which binds within the H3A(+30+50) and H3A(+35+65) annealing sites but does not induce exon skipping.

151. The '350 Patent also includes data refuting the notion that a hot spot can be identified from a small number of ASOs. Dr. Wilton discloses the ability of 10 ASOs to induce exon 43 skipping:

ASO	Ability to Induce Exon Skipping
H43A(-09+18)	Faint skipping to 25 nM
H43A(+81+111)	Strong skipping at 50 nM faint 2.5 nM
H43A(+83+110)	No skipping
H43A(+89+117)	Strong skipping at 25 nM faint 2.5 nM
H43A(+92+114)	Faint skipping to 2.5 nM
H43A(+92+117)	Skipping at 10 nM
H43A(+92+120)	Strong skipping at 10 nM faint 5 nM
H43A(+95+117)	Strong skipping at 25 nM faint 10 nM
H43A(+101+130)	No Skipping
H43D(+08-12)	Skipping down to 200 nM

SRPT-VYDS-0228438 at 489. Dr. Wilton’s description of a hot spot, this data would support the identification of a hot spot between +81 and +120 of Exon 43. This region could have been defined as the region between annealing site H43A(+81+111) (“Strong skipping at 50 nM faint 2.5 nM”) and H43A(+92+120) (“Strong skipping at 10 nM faint 5 nM”). *Id.* Four additional ASOs within this region also induce exon skipping: H43A(+89+117), H43A(+92+114), H43A(+92+117), and H43A(+95+117). *Id.*

152. The data reported for Exon 43 is even more robust than the data Dr. Wilton purports shows that he identified a hot spot in Exon 53. The purported Exon 53 hot spot was identified based on the ability of four ASOs to induce exon skipping in a 47-base pair region. SRPT-VYDS-0002641 at 717. The Exon 43 data in the ’350 Application shows six ASOs that induce exon skipping within a 40-base region. SRPT-VYDS-0228438 at 489. Yet, the data reported for Exon 3 also includes H3A(+37+61), which binds within H3A(+30+50) and H3A(+35+65) annealing sites but does not induce exon skipping. *Id.*

153. The results for the Exon 3 and Exon 43 in the ’851 Patent and ’350 Application, respectively, demonstrate that it is not possible to identify a hot spot from the limited data presented for Exon 53 in the ’851 Patent. Even when the hot spot is relatively small, such as the 36-base region of Exon 3 described above, merely identifying a region with four ASOs that each

induce exon skipping does not guarantee that other ASOs falling within that region will also induce exon skipping.

E. Post-Filing Date Data Provides Further Evidence that UWA Was Not in Possession of a Sufficient Number of Species at the Alleged Priority Date

1. European Opposition Experimental Data

154. I understand that NS challenged a European patent (EP 2206781 B1) at the European Patent Office in an Opposition proceeding, which stems from the same patent application that led to the UWA Patents. Claim 1 of that European patent was directed to similar subject matter as the '851 Patent, i.e., an “antisense oligonucleotide that binds to human dystrophin pre-mRNA wherein said oligonucleotide is 20 to 31 nucleotides in length and is an oligonucleotide that is specifically hybridizable to an exon 53 target region of the Dystrophin gene designated as annealing site H53A (+23+47), annealing site H53A (+39+69), or both.” In response to certain of NS’s arguments in the proceeding, UWA submitted a declaration by Dr. Fred Schnell, dated April 25, 2018 (SRPT-VYDS-0228268), containing post-filing date data of several antisense oligonucleotides that fall within the scope of the UWA Patent claims. Tables 1 and 2 of the Schnell Declaration are reproduced below and provide a summary of antisense oligonucleotides 18 to 23 nucleotides in length that exhibit exon 53 skipping activity:

Table 1: Experiment 1 Exon 53 Skipping Experimental Results

Compound Name	Length	Dose					
		10µM		5µM		2.5µM	
		Mean	SD	Mean	SD	Mean	SD
Comparator H53A(+45+62)	18	2.9	2.7	0.5	0.9	0.6	1.0
AON2 (+32+51)	20	9.6	2.3	6.7	0.6	5.5	2.0
AON2 (+37+56)	20	17.2	4.7	10.8	4.9	5.8	1.1
AON3 (+40+59)	20	7.3	4.1	2.8	2.7	0.9	1.6
AON4(+36+56)	21	20.8	11.5	9.8	3.1	8.2	4.1
AON5 (+23+44)	22	11.3	7.0	5.9	1.1	4.3	2.1
AON6 (+29+50)	22	6.7	0.3	4.4	0.3	2.1	0.8
AON7 (+38+59)	22	2.5	2.3	3.3	0.2	0.8	1.4
AON8 (+41+62)	22	2.4	2.1	2.1	1.8	0.0	0.0
AON9 (+44+65)	22	0.7	1.0	0.0	0.0	0.0	0.0
AON10 (+32+54)	23	10.3	1.5	5.7	1.0	1.8	2.6
AON11 (+47+69)	23	0.0	0.0	0.0	0.0	0.0	0.0

Table 2: Experiment 2 Exon 53 Skipping Experimental Results

Compound Name	Length	Dose					
		10µM		5µM		2.5µM	
		Mean	SD	Mean	SD	Mean	SD
Comparator H53A(+45+62)	18	4.5	2.2	2.2	1.2	0.8	1.1
AON4(+36+56)	21	21.6	4.3	17.5	6.7	16.3	0.2
AON12 (+23+42)	20	13.7	2.3	7.2	2.2	2.1	2.9
AON13 (+26+45)	20	12.7	3.5	4.0	2.2	0.0	0.0
AON14 (+29+48)	20	10.8	0.0	5.2	1.1	2.9	1.2
AON15 (+38+57)	20	3.6	3.2	4.5	4.1	1.0	1.7
AON16 (+41+60)	20	7.4	3.5	2.5	2.4	5.1	3.8
AON17 (+44+63)	20	2.9	2.7	3.7	0.2	1.0	1.8
AON18 (+47+66)	20	0.0	0.0	0.5	0.9	0.0	0.0
AON19 (+23+43)	21	11.2	4.5	8.1	4.0	7.9	1.4
AON20 (+26+46)	21	16.0	24.4	8.2	7.2	1.6	2.2
AON21 (+29+49)	21	7.9	0.4	3.2	0.8	1.1	1.6
AON22 (+32+52)	21	18.4	4.5	5.4	4.8	6.3	2.0
AON23 (+38+58)	21	9.6	5.8	0.0	0.0	3.8	0.3
AON24 (+41+61)	21	5.5	1.6	1.7	3.0	1.2	2.0
AON25 (+44+64)	21	0.6	1.0	0.0	0.0	0.0	0.0
AON26 (+46+66)	21	0.0	0.0	0.0	0.0	1.4	2.4
AON27 (+31+53)	23	10.6	5.6	6.4	6.0	2.8	4.0
AON28 (+36+58)	23	15.2	3.1	4.8	4.9	5.2	2.0
AON29 (+39+61)	23	3.3	3.1	2.6	2.3	2.5	2.8
AON30 (+40+62)	23	5.9	1.3	3.4	3.9	2.4	4.1
AON31 (+45+67)	23	0.0	0.0	0.0	0.0	0.0	0.0
AON32 (+46+68)	23	0.6	1.0	3.0	2.6	0.0	0.0

155. Several of the antisense oligonucleotides that Dr. Schnell tested fall within the scope of the UWA Patent claims, including antisense oligonucleotides 2, 4, 6, 10, 14, 18, 21, 22, 27 and 28. It is my understanding that such post-filing data, however, cannot be used to affirmatively satisfy the written description requirement as written description requires a specification to reasonably convey to those skilled in the art that the inventor had possession of the claimed subject matter as of the alleged priority date. The data in the Schnell declaration is not disclosed in the UWA Patents, and therefore, cannot convey possession of the claimed subject matter as of the alleged priority date. The fact that UWA was compelled to test additional species after-the-fact in the European Opposition proceeding further shows that the inventors failed to disclose a representative number of species in the specification of the UWA Patents.

2. Chemicals Evaluation and Research Institute (CERI) Experimental Reports

156. While evaluating the sufficiency of the UWA Patents' written description and enablement, I considered whether additional testing would be informative to the inquiries, *e.g.*, the disclosure of a representative number of species, the existence of a structure-function relationship and the predictability of exon-skipping antisense oligonucleotides directed to exon 53. I have been informed by counsel and understand that, although the written description inquiries apply the lens of a POSA at the time of the inventions, post-priority date testing may be relied upon as evidence of such factors.

157. Accordingly, I designed protocols for experiments ultimately performed by CERI at my direction, which are described in the three CERI Reports. *See generally* NS00102924 (Study Number: 936-21-M-0643); NS00102988 (Study Number: 936-21-M-0644); NS00103061 (Study Number: 936-22-M-0661).

158. CERI was chosen to conduct these experiments because it was qualified to conduct them and because my laboratory at Rosalind Franklin University (where I worked when I designed these experiments) did not have the resources to conduct these experiments in addition to our other ongoing research. Like many contract research organizations, CERI offers “state-of-the-art facilities” and “highly qualified professionals” who “conduct a range of chemical analysis techniques and bio-testing methods”:

As an independent, unbiased organization that is committed to keeping chemicals and the environment in balance, CERI plays a supporting role in the research and development projects of clients utilizing its services. Working in state-of-the-art facilities, our highly qualified professionals conduct a range of chemical analysis techniques and bio-testing methods for these clients. CERI’s staff has also performed tests, analysis, research, and studies designed to solve technical problems in the field of chemicals.

See https://www.cerij.or.jp/ceri_en/gaiyou/gaiyou_menu.html. The experiments I designed were carried out by CERI’s Chemicals Assessment and Research Center, which has experience developing “novel methodology” relating to “cell based assay and other in vitro methodologies,” as well as performing “[j]oint research with partners in industry, academia, and the government.” See https://www.cerij.or.jp/ceri_en/gyoumu/hyoukaken_menu.html. In my opinion, this made CERI well-suited for this type of research project, which involved implementing custom experimental protocols I designed.

159. CERI had suitable equipment and could source suitable reagents for the experimental protocols I designed, each of which is described in the “Materials and Methods” section of the corresponding CERI Report. To ensure control of starting materials, my protocols called for using commercially available cells instead of any cell-lines it had on-hand. Cells were therefore sourced from the manufacturer Lonza and provided to CERI.



a. Study Number: 936-21-M-0643

160. Study No. 936-21-M-0643 (NS00102924) relates to testing 2'-O-methyl antisense oligonucleotides. I decided to conduct this testing because, in my opinion, a POSA would find the specification lacking for actual data on SEQ ID No. 195 and other exon 53-directed ASOs. Apart from SEQ ID NO: 193, the figures do not show any testing of individual exon 53-directed antisense oligonucleotides. '851 Patent at Figure 22. In my opinion, a POSA reviewing the specification would understand Table 39 as reporting on the activity of the individual antisense oligonucleotides listed. However, the only objective data provided for SEQ ID NO: 195 is as part of a cocktail with SEQ ID NOs: 194 and 196.²⁶ *Id.* at Figure 22; 64:41-45. Although the specification discusses exon 53 cocktails and weasels separately in Table 1B and Table 1C, Table 39 reports the exact same “[a]bility to induce skipping” for each antisense oligonucleotide used in the cocktail test: SEQ ID NOs: 194, 195 and 196:

TABLE 39

Antisense oligonucleotide	Sequence	Ability to induce skipping
SEQ ID name		
	*	*

²⁶ The specification's disclosures concerning exon 53 antisense oligonucleotides are rife with errors. The specification, for example, states that Figure 22 shows testing of “H53A(+39+69) [SEQ ID NO:193],” but Figure 22 mislabels this ASO as “H53D(+39+69).” *See* '851 Patent at 64:38-41. The specification's text also describes “H53A(+39+69) [SEQ ID NO:193]” as being tested at a 5 nM concentration, while Table 39 only describes the “[a]bility to induce skipping” down “to 50 nM.” *See id.* Additionally, the specification describes the cocktail test as being shown by Figure 20, but Figure 20 is labeled as relating to H42A(-4+23). *See id.* at 64:41-45, Fig. 20. Figure 22 appears to show the “Cocktail” test for exon 53, but the antisense oligonucleotides are all labeled as “H53D,” when SEQ ID NOs: 195 and 196 should be “H53A.” The extent of these errors would further detract from a POSA's confidence in the robustness of the results reported. In my experience in this field, such extensive, glaring errors in reporting one's results would be unacceptable for peer-reviewed literature and would have to be corrected before publication.

194	H53D (+14-07)	UAC UAA CCU UGG UUU CUG UGA	Very faint skipping to 50 nM
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C	Very faint skipping to 50 nM
196	H53A (+150+176)	UGU AUA GGG ACC CUC CUU CCA UGA CUC	Very faint skipping to 50 nM

161. This led me to question whether the activity reported in Table 39 for SEQ ID NOs: 194, 195 and 196, was based on testing the cocktail discussed in the specification. I therefore designed an experimental protocol to test those three antisense oligonucleotides both individually and as a cocktail.²⁷ Because my goal was to evaluate the UWA Patents' disclosures with this experiment, I selected the same antisense oligonucleotide backbone (2'-O-methyl) as used in the UWA Patent examples. *See, e.g.*, '851 Patent at 32:33-47 ("2OMe antisense oligonucleotides were designed to be complementary to the target sequences under investigation and were synthesized on an Expedite 8909 Nucleic Acid Synthesiser. . . . Specified amounts of the antisense molecules were then tested for their ability to induce exon skipping in an in vitro assay, as described below."). Likewise, I selected normal human muscle cells because the specification's examples did so. *Id.* at 32:48-50. The selected concentrations (300 nM and 600 nM) of 2'-O-methyl antisense oligonucleotides were also used in the specification's examples for 2'-O-methyl antisense oligonucleotides for exon 53. *See id.* at 64:32-50.

162. The specification generically states that the named inventors performed cell culturing, transfection, RNA extraction, and RT-PCR to obtain their data, but does not describe

²⁷ In my experience, seeking to replicate another's previously-reported results is commonplace in scientific fields as a confirmatory check on data. *See also* Adams Dep. Tr. at 32:2-14

any of the experimental procedures or techniques employed that would enable a POSA to replicate them exactly. *See, e.g.*, '851 Patent at 32:48-60. I therefore modeled my experimental protocol largely after the named inventors' methods as described in Dr. Wilton's and Dr. Fletcher's publications from around the year 2005, namely Errington, Stephen J., et al. "Target selection for antisense oligonucleotide induced exon skipping in the dystrophin gene." *The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications* 5.6 (2003): 518-527, and Harding. Where necessary, I used my professional judgment to select appropriate modifications, such as in identifying suitable reagents to replace those identified in Errington or Harding that were no longer commercially available. As one example, I decided to use the RT-PCR primers reported in Sarepta's U.S. Patent Application Publication No. 2010/0130591 A1 to Sazani and Kole for detecting exon 53 skipping in human dystrophy (*see* tbl. 1) instead of those reported in Harding because they generate shorter PCR products. The percent skipping for each PCR product was calculated by the following formula:

$$\% \text{ Skipping} = \frac{\text{Skipped [153 nt band]} \times D}{\text{Skipped [153 nt band]} \times D + \text{Full Length [365 nt band]} \times D} \times 100$$

NS00102937.²⁸

163. A summary of the tested 2'-O-methyl antisense oligonucleotides is provided in the table below. I selected the UWA Patents' self-described "strongest" antisense oligonucleotide at inducing exon 53 skipping (SEQ ID NO: 195) as the positive control. *See* '851 Patent at 64:49-50. The specification did not identify any antisense oligonucleotide negative controls, so I used "blanks" (*i.e.*, 0 nM concentrations) as a negative control.

²⁸ The same formula was used in each of the three CERI reports.

Tested ASO ²⁹	Length	Position on Exon 53	Skipping	Notes
#11 (ASO-1)	31 mer	+39+69	Yes	Positive Control SEQ ID NO: 193
#13 (ASO-3)	21 mer	+199+219 ³⁰	No	SEQ ID NO: 194
#14 (ASO-4)	25 mer	+23+47	Yes ³¹	SEQ ID NO: 195
#15 (ASO-5)	27 mer	+150+176	No	SEQ ID NO: 196
Cocktail (ASO-3, -4, -5)	<i>See above</i>	<i>See above</i>	Yes	<i>See above</i>

164. As shown in the tables below, this experimental protocol detected exon 53-skipping for SEQ ID NO: 195 and the cocktail, but SEQ ID NOs: 194 and 196 induced little to no skipping, even at the higher 600 nM concentration. These individual results (which vary across the three cocktail antisense oligonucleotides) are inconsistent with how Table 39 of the UWA Patents describes their “Ability to induce skipping” (identically across all three as “Very faint skipping to 50 nM”).

165. My results are not inconsistent with a conclusion that Table 39 reports the activity of a SEQ ID NOs: 194, 195, and 196 cocktail, *not* each antisense oligonucleotide tested individually. If a POSA conducted this confirmatory experiment and obtained the results that I did, they might conclude that the exon skipping activity of SEQ ID NO: 195 reported in Table 39 was based on its performance in a cocktail.³² This would further support my opinion that a POSA would not understand the named inventors to have possessed the claimed genus of PMOs at the

²⁹ ASO = antisense oligonucleotide

³⁰ If listed relative to the exon 53 donor site, the coordinates would be H53D(+14-07).

³¹ The results for ASO-4 are not consistent with the results reported in Table 39 of the UWA Patents for SEQ ID NO: 195.

. See Section IX.D., above.

³²

time of the invention. Because the activity of individual antisense oligonucleotides within a cocktail cannot be determined or predicted from the activity of the cocktail, a POSA would *not* understand that SEQ ID NO: 195 induces exon-skipping individually based on the activity of the cocktail. *See, e.g.*, Adams 2007 (“Some AOs were inactive when applied individually, yet pronounced exon excision was induced in transfected cells when the AOs were used in select combinations”). As such, the specification would certainly not provide a POSA any basis to conclude that the inventors possessed an entire genus of exon 53-skipping PMOs having as few as a mere 12 consecutive bases of SEQ ID NO: 195 based on the “faint skipping at 50 nM” induced by a “cocktail” containing SEQ ID NO: 195.

166. A more detailed summary of the results obtained from this study is presented in the tables below.

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
ASO-1	0 nmol/L	1	374	2,420	100	(38)	n.d.	n.d.	1	(37)	N/A
		2	376	1,620	100	(38)	n.d.	n.d.	1	(37)	N/A
		3	375	2,350	100	(38)	n.d.	n.d.	1	(37)	N/A
	300 nmol/L	1	374	1,050	100	(38)	162	638	100	(38)	37.8
		2	374	1,030	100	(38)	161	726	100	(38)	41.3
		3	372	1,040	100	(38)	161	705	100	(38)	40.4
	0 nmol/L	1	374	2,060	100	(40)	n.d.	n.d.	1	(39)	N/A
		2	372	1,780	100	(40)	n.d.	n.d.	1	(39)	N/A
		3	369	1,820	100	(40)	n.d.	n.d.	1	(39)	N/A
	600 nmol/L	1	371	664	100	(40)	161	819	100	(40)	55.2
		2	369	647	100	(40)	161	995	100	(40)	60.6

	3	366	632	100	(40)	161	784	100	(40)	55.4
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n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
ASO-3	0 nmol/L	1	374	2,420	100	(42)	n.d.	n.d.	1	(41)	N/A
		2	376	1,620	100	(42)	n.d.	n.d.	1	(41)	N/A
		3	375	2,350	100	(42)	n.d.	n.d.	1	(41)	N/A
	300 nmol/L	1	370	2,200	100	(42)	154	2,570	1	(41)	1.2
		2	376	1,830	100	(42)	157	1,430	1	(41)	0.8
		3	378	2,110	100	(42)	157	1,570	1	(41)	0.7
	0 nmol/L	1	374	2,060	100	(43)	n.d.	n.d.	1	(45)	N/A
		2	372	1,780	100	(43)	n.d.	n.d.	1	(45)	N/A
		3	369	1,820	100	(43)	n.d.	n.d.	1	(45)	N/A
	600 nmol/L	1	382	1,660	100	(44)	148	1,080	1	(45)	0.7
		2	382	1,510	100	(44)	155	297	1	(45)	0.2
		3	381	1,690	100	(44)	148	1,290	1	(45)	0.8

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
ASO-4	0 nmol/L	1	374	2,420	100	(47)	n.d.	n.d.	1	(46)	N/A
		2	376	1,620	100	(47)	n.d.	n.d.	1	(46)	N/A
		3	375	2,350	1.00	(47)	n.d.	n.d.	1	(46)	N/A
	300 nmol/L	1	377	1,500	100	(47)	164	48.5	100	(47)	24.4
		2	376	1,420	100	(47)	161	514	100	(47)	26.6

	3	374	1,200	100	(47)	160	604	100	(47)	33.5
	1	374	2,060	100	(49)	n.d.	n.d.	1	(48)	N/A
0 nmol/L	2	372	1,780	100	(49)	n.d.	n.d.	1	(48)	N/A
	3	369	1,820	100	(49)	n.d.	n.d.	1	(48)	N/A
	1	382	1,090	100	(50)	165	522	100	(50)	32.4
600 nmol/L	2	382	998	100	(50)	164	649	100	(50)	39.4
	3	381	825	100	(50)	164	579	100	(50)	41.2

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
		1	374	2,420	100	(52)	n.d.	n.d.	1	(51)	N/A
	0 nmol/L	2	376	1,620	100	(52)	n.d.	n.d.	1	(51)	N/A
		3	375	2,350	100	(52)	n.d.	n.d.	1	(51)	N/A
		1	370	2,400	100	(52)	154	419	1	(51)	0.2
	300 nmol/L	2	368	2,200	100	(52)	154	489	1	(51)	0.2
		3	366	2,450	100	(52)	153	468	1	(51)	0.2
ASO-5		1	374	2,060	100	(54)	n.d.	n.d.	1	(53)	N/A
	0 nmol/L	2	372	1,780	100	(54)	n.d.	n.d.	1	(53)	N/A
		3	369	1,820	100	(54)	n.d.	n.d.	1	(53)	N/A
		1	380	1,380	100	(55)	154	2,190	1	(53)	1.6
	600 nmol/L	2	385	1,410	100	(55)	149	1,050	1	(53)	0.7
		3	388	1,460	100	(55)	148	4,840	1	(53)	3.2

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size	Conc.	Dilution	Fig.	Size	Conc.	Dilution	Fig.	

		(bp)	(pmol/L)	factor		(bp)	(pmol/L)	factor		
	1	374	2,420	100	(57)	n.d.	n.d.	1	(56)	N/A
	0 nmol/L	2	376	1,620	100	(57)	n.d.	n.d.	1	(56) N/A
	3	375	2,350	100	(57)	n.d.	n.d.	1	(56)	N/A
	1	381	3,700	20	(58)	171	2,150	20	(58)	36.8
	300 nmol/L	2	385	2,600	20	(58)	174	811	20	(58) 23.8
	3	384	3,400	20	(58)	171	1,900	20	(58)	35.8
Mixture of ASO- 3, ASO-4, and ASO- 5 (1 st Run)	1	374	2,060	100	(60)	n.d.	n.d.	1	(59)	N/A
	0 nmol/L	2	372	1,780	100	(60)	n.d.	n.d.	1	(59) N/A
	3	369	1,820	100	(60)	n.d.	n.d.	1	(59)	N/A
	1	387	2,220	20	(61)	172	2,280	20	(61)	50.7
	600 nmol/L	2	384	2,370	20	(61)	170	2,140	20	(61) 47.5
	3	384	2,060	20	(61)	170	2,980	20	(61)	59.1

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Size (bp)	Conc. (pmol/L)	Dilution factor		
		1	374	2,420	100	(57)	n.d.	n.d.	1	(56) N/A
	0 nmol/L	2	376	1,620	100	(57)	n.d.	n.d.	1	(56) N/A
	3	375	2,350	100	(57)	n.d.	n.d.	1	(56)	N/A
	1	384	3,100	20	(64)	170	3,840	20	(64)	55.3
	300 nmol/L	2	390	3,350	20	(64)	174	3,780	20	(64) 53.0
	3	380	3,500	20	(64)	173	2,560	20	(64)	42.2
Mixture of ASO- 3, ASO-4, and ASO- 5 (2 nd Run)	1	374	2,060	100	(60)	n.d.	n.d.	1	(59)	N/A
	0 nmol/L	2	372	1,780	100	(60)	n.d.	n.d.	1	(59) N/A
	3	369	1,820	100	(60)	n.d.	n.d.	1	(59)	N/A

	1	383	1,230	20	(67)	167	3,880	20	(67)	75.9
600 nmol/L	2	381	1,050	20	(67)	167	4,410	20	(67)	80.8
	3	375	910	20	(67)	164	4,170	20	(67)	82.1

n.d.: not detected, N/A: Not Applicable

b. Study Numbers: 936-21-M-0644 and 936-22-M-0661

167. Study Nos. 936-21-M-0644 (NS00102988) and 936-22-M-0661 (NS00103061) relate to testing PMOs. I designed these experiments to provide more data regarding whether and how exon skipping activity varies across the variety of different PMOs with different chemical structures. The PMOs I selected each are between 20 to 31 bases in length, include at least 12 consecutive bases of SEQ ID NO: 195 that is 100% complementary to a segment of pre-mRNA transcribed from exon 53 of the human dystrophin gene, and contain only thymine bases (no uracil bases).

168. To assess the breadth of the claims, I selected PMOs having different sets of at least 12 consecutive bases of SEQ ID NO: 195 and also designed the remainder of the PMOs in a variety of ways, including (1) having additional bases complementary to segment of exon 53 immediately adjacent to the bases to which the core “base sequence” is complementary; (2) having additional bases complementary to a segment of exon 53 not adjacent to the bases to which the core “base sequence” is complementary; (3) having additional random bases on one or both ends of the core “base sequence” that may or may not be complementary to exon 53; and (4) having additional bases with sequences that are self-complementary to the core “base sequence.” As discussed below, these results provided helpful information demonstrating the unpredictability of exon-skipping across PMOs and the lack of structure-function relationship between exon 53-skipping and the claimed structural features.

169. Because my goal was to evaluate the claimed genus, I selected the antisense oligonucleotide backbone (PMO) claimed. I selected normal human muscle cells because the specification's examples did so. *See* '851 Patent at 32:48-50. To ensure robustness of my results, I decided to test the PMOs at nanomolar concentrations (300 nM and 600 nM) used in the specification's examples and at a higher micromolar concentration (10 μ M) concentration I have more commonly seen used for PMOs.

170. Again, I modeled my experimental protocol largely after the named inventors' methods as described in Dr. Wilton's and Dr. Fletcher's publications from near 2005, as noted above (Study No. 936-21-M-0643). Where necessary, I used my professional judgment to select appropriate modifications, such as in identifying suitable reagents to replace those identified in Errington or Harding that were no longer commercially available. As an example, I decided to use a more modern transfection technique (with Endo-Porter) than that disclosed in Harding that I felt would better ensure that the PMOs would be delivered to cells (*i.e.*, to avoid false negatives).³³ Again, I decided to use the RT-PCR primers reported in Sarepta's U.S. Patent Application Publication No. 2010/0130591 A1 to Sazani and Kole for detecting exon 53 skipping in human dystrophy (*see* Table 1) instead of those reported in Harding, because they would provide a shorter PCR product.

(1) Study No. 936-21-M-0644

171. I selected a PMO targeting +39+62 as a positive control based on the UWA Patents' assertion that an AON targeting this region has "faint skipping at 50 nM," *see* '851 Patent at Table

³³ I also consulted Summerton, James, and Dwight Weller. "Morpholino antisense oligomers: design, preparation, and properties." *Antisense and Nucleic Acid Drug Development* 7.3 (1997): 187-195, and considered its scrape-loading method, but ultimately decided to proceed with the more modern Endo Porter technique.

39, and because obtaining a 31-mer PMO from a vendor would have been practically difficult. PMOs-3 (-12+10), -4 (-7+18), -5 (+7+26), and -6 (+124+145), all of which do not fall within the scope of the UWA Patent claims and are reported by UWA to not induce skipping (*see id.*), were selected as negative controls.

172. PMOs-7, -8, -10 and -11 were designed to include 12 consecutive bases of SEQ ID NO: 195, i.e., the core “base sequence,” that hybridizes to position +23+34 of exon 53. Each PMO was also designed to include a sequence of bases added to the 3’ end of the PMO. PMO-7 includes 18 bases, of which 3 bases are complimentary to positions 5, 8 and 20 of exon 53, and the remaining 15 are random bases. PMO-8 also includes 18 additional bases, of which 6 are random bases and 12 form a sequence of bases that is self-complimentary to the 12 consecutive bases of SEQ ID NO: 195 that hybridize to position +23+34 of exon 53. PMO-10 includes 8 additional bases that form a sequence of bases that is self-complimentary to 8 consecutive bases of SEQ ID NO: 195 that hybridize to position +27+34. PMO-11 includes 8 additional bases, of which 7 are random and 1 is complimentary to position 16 of exon 53. Importantly, PMOs-7, -8, -10 and -11 all meet all structural requirements of the claims, other than being 100% complementary to the dystrophin pre-mRNA.³⁴

Study No. 936-21-M-0644				
Tested PMO	Length	Position on Exon 53	Skipping	Notes
#22 (PMO-2)	24 mer	+39+62	Yes	Positive Control
#24 (PMO-3)	22 mer	-12+10	No	Negative Control
#25 (PMO-4)	25 mer	-7+18	No	Negative Control
#26 (PMO-5)	20 mer	+7+26	No	Negative Control

³⁴ These PMOs had previously met all structural requirements under the Court’s prior construction.

#27 (PMO-6)	22 mer	+124+145	No	Negative Control
#28 (PMO-7)	30 mer	+23+34	No	Experimental
#29 (PMO-8)	30 mer	+23+34	No	Experimental
#30 (PMO-9)	30 mer	+36+65	Yes	Experimental
#31 (PMO-10)	20 mer	+23+34	No	Experimental
#32 (PMO-11)	20 mer	+23+34	No	Experimental
#33 (PMO-12)	25 mer	+10+34	Yes	Experimental
#34 (PMO-13)	25 mer	+23+47	Yes	Experimental
#35 (PMO-14)	20 mer	+23+42	Yes	Experimental
#36 (PMO-15)	20 mer	+28+47	Yes	Experimental
#37 (PMO-16)	30 mer	+23+52	Yes	Experimental
#38 (PMO-17)	20 mer	+48+67	No	Experimental

173. As summarized above, PMOs that hybridize to position +23+34 on exon 53, *i.e.*, comprising “at least 12 consecutive bases” of SEQ ID NO: 195 and include additional random and/or self-complimentary bases (PMOs-7, -8, -10 and -11) induced little to no skipping at all of the tested concentrations, while a PMO that hybridizes to position +36+65 on exon 53 (PMO-9) (comprising 12 consecutive bases of SEQ ID NO: 195 that are different from those in PMOs-7, -8, -10 and -11) induced skipping at both 600 nM and 10 μ M. Moreover, PMOs comprising the same “12 consecutive bases” of SEQ ID NO: 195 (targeting position +23+34) did not uniformly induce exon 53 skipping. *Compare* PMOs-13, -14 and 16 *with* PMOs-7, -8, -10 and -11.

174. In my opinion, these data supports my opinions above in several ways. First, because these experiments show varying levels of activity (including no activity in certain PMOs) when testing a variety of morpholino ASOs with thymine bases and different sets of “at least 12 consecutive bases of SEQ ID NO: 195,” they generally confirm the unpredictability of achieving exon skipping in exon 53 and that having 12 consecutive bases will not reliably induce exon skipping. Further, because each PMO tested was complementary to at least the seven base region (h53A(+36+42)) shared by all claimed ASOs of the ’851 Patent, they contradict the notion that

simply targeting that region with morpholino ASOs of 20-31 bases having thymine bases would reliably and predictably induce exon 53 skipping.

175. A more detailed summary of the results obtained from this study is presented in the tables below.

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
PMO-2	0 nmol/L	1	378	2,990	40	(3)	n.d.	n.d.	1	(2)	N/A
		2	376	2,980	40	(3)	n.d.	n.d.	1	(2)	N/A
		3	375	2,800	40	(3)	n.d.	n.d.	1	(2)	N/A
	300 nmol/L	1	375	2,540	40	(3)	159	482	1	(2)	0.5
		2	373	2,580	40	(3)	157	535	1	(2)	0.5
		3	372	2,450	40	(3)	157	816	1	(2)	0.8
	600 nmol/L	1	367	2,860	40	(3)	156	631	1	(2)	0.5
		2	369	2,610	40	(3)	163	223	40	(3)	7.9
		3	365	3,000	40	(3)	162	232	40	(3)	7.2
	10 μ mol/L	1	384	1,790	40	(4)	169	617	40	(4)	25.6
		2	387	1,670	40	(4)	169	614	40	(4)	26.9
		3	390	1,660	40	(4)	170	567	40	(4)	25.5

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
PMO-3	0 nmol/L	1	378	2,990	40	(8)	n.d.	n.d.	1	(5)	N/A
		2	376	2,980	40	(8)	n.d.	n.d.	1	(5)	N/A
		3	375	2,800	40	(8)	n.d.	n.d.	1	(5)	N/A
	300 nmol/L	1	383	2,260	40	(9)	n.d.	n.d.	1	(6)	N/A

	2	383	2,500	40	(9)	n.d.	n.d.	1	(6)	N/A
	3	383	2,350	40	(9)	n.d.	n.d.	1	(6)	N/A
	1	381	2,810	40	(9)	n.d.	n.d.	1	(6)	N/A
600 nmol/L	2	381	2,670	40	(9)	158	660	1	(7)	0.6
	3	381	2,390	40	(9)	158	241	1	(6)	0.3
	1	373	1,540	60	(10)	159	261	1	(6)	0.3
10 µmol/L	2	382	1,950	40	(9)	164	388	1	(6)	0.5
	3	381	1,760	40	(9)	165	243	1	(6)	0.3

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
		1	378	1,940	40	(12)	n.d.	n.d.	1	(11)	N/A
	0 nmol/L	2	378	1,680	40	(12)	n.d.	n.d.	1	(11)	N/A
		3	375	1,690	40	(12)	n.d.	n.d.	1	(11)	N/A
		1	376	1,880	40	(12)	n.d.	n.d.	1	(11)	N/A
	300 nmol/L	2	372	1,880	40	(12)	n.d.	n.d.	1	(11)	N/A
		3	372	1,730	40	(12)	n.d.	n.d.	1	(11)	N/A
PMO-4		1	370	1,970	40	(12)	n.d.	n.d.	1	(11)	N/A
	600 nmol/L	2	388	1,960	40	(13)	n.d.	n.d.	1	(11)	N/A
		3	389	1,720	40	(13)	n.d.	n.d.	1	(11)	N/A
		1	388	2,010	40	(13)	155	302	1	(11)	0.4
	10 µmol/L	2	388	1,700	40	(13)	152	363	1	(11)	0.5
		3	383	1,360	40	(13)	153	547	1	(11)	1.0

n.d.: not detected, N/A: Not Applicable

Exposure	Exposure	replicate	Full length fragment				Skipped fragment			
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Sample	conc.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency	
PMO-5	0 nmol/L	1	378	1,940	40	(16)	n.d.	n.d.	1	(14)	N/A
		2	378	1,680	40	(16)	n.d.	n.d.	1	(14)	N/A
		3	375	1,690	40	(16)	n.d.	n.d.	1	(14)	N/A
	300 nmol/L	1	381	3,230	40	(17)	n.d.	n.d.	1	(14)	N/A
		2	383	2,420	40	(17)	n.d.	n.d.	1	(14)	N/A
		3	386	1,630	40	(17)	n.d.	n.d.	1	(14)	N/A
	600 nmol/L	1	387	1,710	40	(17)	n.d.	n.d.	1	(15)	N/A
		2	382	1,950	40	(17)	n.d.	n.d.	1	(15)	N/A
		3	386	1,580	40	(17)	n.d.	n.d.	1	(15)	N/A
	10 μmol/L	1	383	1,620	40	(17)	n.d.	n.d.	1	(15)	N/A
		2	381	1,340	40	(17)	n.d.	n.d.	1	(15)	N/A
		3	380	1,400	40	(17)	n.d.	n.d.	1	(15)	N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
PMO-6	0 nmol/L	1	390	1,940	40	(20)	n.d.	n.d.	1	(18)	N/A
		2	390	2,380	40	(20)	n.d.	n.d.	1	(18)	N/A
		3	389	2,190	40	(20)	n.d.	n.d.	1	(18)	N/A
	300 nmol/L	1	384	2,020	40	(20)	n.d.	n.d.	1	(19)	N/A
		2	383	1,770	40	(20)	n.d.	n.d.	1	(19)	N/A
		3	379	1,450	40	(20)	n.d.	n.d.	1	(19)	N/A
	600 nmol/L	1	387	2,030	40	(21)	n.d.	n.d.	1	(19)	N/A
		2	387	1,960	40	(21)	n.d.	n.d.	1	(19)	N/A
		3	387	1,840	40	(21)	160	151	1	(19)	0.2

	1	384	1,820	40	(21)	162	100	1	(19)	0.1
10 μ mol/L	2	385	1,640	40	(21)	n.d.	n.d.	1	(19)	N/A
	3	383	2,040	40	(21)	n.d.	n.d.	1	(19)	N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-7	0 nmol/L	1	390	1,940	40	(24)	n.d.	n.d.	1	(22)	N/A
		2	390	2,380	40	(24)	n.d.	n.d.	1	(22)	N/A
		3	389	2,190	40	(24)	n.d.	n.d.	1	(22)	N/A
	300 nmol/L	1	389	1,850	40	(24)	n.d.	n.d.	1	(22)	N/A
		2	387	2,080	40	(24)	n.d.	n.d.	1	(22)	N/A
		3	387	2,430	40	(24)	158	114	1	(22)	0.1
	600 nmol/L	1	383	2,840	40	(24)	154	250	1	(22)	0.2
		2	390	2,490	40	(24)	148	125	1	(22)	0.1
		3	389	2,000	40	(24)	n.d.	n.d.	1	(23)	N/A
	10 μ mol/L	1	390	1,950	40	(24)	154	134	1	(23)	0.2
		2	388	1,740	40	(24)	163	411	1	(23)	0.6
		3	387	2,300	40	(24)	160	228	1	(23)	0.2

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-8	0 nmol/L	1	383	2,250	40	(27)	n.d.	n.d.	1	(25)	N/A
		2	378	2,740	40	(27)	n.d.	n.d.	1	(25)	N/A
		3	379	2,060	40	(27)	n.d.	n.d.	1	(25)	N/A
	300 nmol/L	1	379	4,010	40	(28)	n.d.	n.d.	1	(25)	N/A

	2	379	1,680	40	(28)	n.d.	n.d.	1	(25)	N/A
	3	380	2,180	40	(28)	n.d.	n.d.	1	(25)	N/A
	1	383	1,700	40	(28)	n.d.	n.d.	1	(26)	N/A
600 nmol/L	2	382	2,360	40	(28)	n.d.	n.d.	1	(26)	N/A
	3	381	2,100	40	(28)	n.d.	n.d.	1	(26)	N/A
	1	379	1,570	40	(28)	n.d.	n.d.	1	(26)	N/A
10 µmol/L	2	379	1,600	40	(28)	n.d.	n.d.	1	(26)	N/A
	3	377	1,210	40	(28)	n.d.	n.d.	1	(26)	N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
		1	383	2,250	40	(30)	n.d.	n.d.	1	(29)	N/A
	0 nmol/L	2	378	2,740	40	(30)	n.d.	n.d.	1	(29)	N/A
		3	379	2,060	40	(30)	n.d.	n.d.	1	(29)	N/A
		1	380	1,670	40	(30)	158	427	1	(29)	0.6
	300 nmol/L	2	379	2,020	40	(30)	162	475	1	(29)	0.6
		3	378	1,910	40	(30)	159	495	1	(29)	0.6
PMO-9		1	373	2,790	40	(30)	165	130	40	(30)	4.5
	600 nmol/L	2	368	2,340	40	(30)	162	158	40	(30)	6.3
		3	384	2,210	40	(31)	169	98.8	40	(31)	4.3
		1	386	1,550	40	(31)	166	724	40	(31)	31.4
	10 µmol/L	2	386	1,420	40	(31)	166	697	40	(31)	32.9
		3	382	1,380	40	(31)	164	756	40	(31)	35.4

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size	Conc.	Dilution	Fig.	Size	Conc.	Dilution	Fig.	

		(bp)	(pmol/L)	factor		(bp)	(pmol/L)	factor		
PMO-10	0 nmol/L	1	388	2,390	40	(34)	n.d.	n.d.	1	(32) <i>N/A</i>
		2	387	2,570	40	(34)	n.d.	n.d.	1	(32) <i>N/A</i>
		3	385	1,770	40	(34)	n.d.	n.d.	1	(32) <i>N/A</i>
	300 nmol/L	1	388	1,310	60	(35)	n.d.	n.d.	1	(33) <i>N/A</i>
		2	387	1,050	60	(35)	n.d.	n.d.	1	(33) <i>N/A</i>
		3	386	1,450	60	(35)	n.d.	n.d.	1	(33) <i>N/A</i>
	600 nmol/L	1	386	3,050	40	(36)	n.d.	n.d.	1	(33) <i>N/A</i>
		2	390	1,880	40	(36)	n.d.	n.d.	1	(33) <i>N/A</i>
		3	388	1,730	40	(36)	n.d.	n.d.	1	(33) <i>N/A</i>
	10 µmol/L	1	384	1,640	40	(36)	n.d.	n.d.	1	(33) <i>N/A</i>
		2	384	1,370	40	(36)	n.d.	n.d.	1	(33) <i>N/A</i>
		3	388	1,770	40	(36)	n.d.	n.d.	1	(33) <i>N/A</i>

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
PMO-11	0 nmol/L	1	382	2,480	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
		2	378	2,350	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
		3	375	1,680	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
	300 nmol/L	1	374	2,250	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
		2	374	2,040	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
		3	371	2,040	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
	600 nmol/L	1	366	2,140	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
		2	366	2,530	40	(38)	n.d.	n.d.	1	(37)	<i>N/A</i>
		3	386	1,910	40	(39)	n.d.	n.d.	1	(37)	<i>N/A</i>

	1	390	2,280	40	(39)	n.d.	n.d.	1	(37)	N/A
10 μ mol/L	2	390	1,790	40	(39)	156	238	1	(37)	0.3
	3	384	3,370	40	(39)	n.d.	n.d.	1	(37)	N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
		1	382	2,480	40	(43)	n.d.	n.d.	1	(40)	N/A
	0 nmol/L	2	378	2,350	40	(43)	n.d.	n.d.	1	(40)	N/A
		3	375	1,680	40	(43)	n.d.	n.d.	1	(40)	N/A
		1	385	3,790	40	(44)	n.d.	n.d.	1	(40)	N/A
	300 nmol/L	2	386	3,810	40	(44)	n.d.	n.d.	1	(40)	N/A
		3	384	3,190	40	(44)	154	266	1	(40)	0.2
PMO-12		1	384	1,580	60	(45)	177	87.1	40	(41)	3.5
	600 nmol/L	2	391	2,340	40	(44)	n.d.	n.d.	1	(42)	N/A
		3	391	2,280	40	(44)	152	441	1	(42)	0.5
		1	387	3,880	40	(44)	171	419	40	(44)	9.7
	10 μ mol/L	2	383	3,120	40	(44)	169	385	40	(44)	11.0
		3	384	3,310	40	(44)	169	334	40	(44)	9.2

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
		1	388	2,390	40	(50)	n.d.	n.d.	1	(46)	N/A
	0 nmol/L	2	387	2,570	40	(50)	n.d.	n.d.	1	(46)	N/A
		3	385	1,770	40	(50)	n.d.	n.d.	1	(46)	N/A
PMO-13	300 nmol/L	1	383	1,600	40	(50)	159	325	1	(46)	0.5

	2	388	2,390	40	(50)	157	566	1	(47)	0.6
	3	385	1,690	40	(50)	153	507	1	(46)	0.7
	1	388	791	100	(51)	167	229	40	(48)	10.4
600 nmol/L	2	379	970	100	(52)	169	107	100	(52)	9.9
	3	390	2,150	40	(50)	153	514	1	(49)	0.6
	1	391	1,280	40	(50)	168	665	40	(50)	34.2
10 μ mol/L	2	391	2,000	40	(50)	169	829	40	(50)	29.3
	3	390	2,040	40	(50)	169	873	40	(50)	30.0

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
		1	376	1,250	100	(58)	n.d.	n.d.	1	(53)	<i>N/A</i>
	0 nmol/L	2	390	1,720	60	(59)	n.d.	n.d.	1	(53)	<i>N/A</i>
		3	388	2,390	40	(56)	n.d.	n.d.	1	(53)	<i>N/A</i>
		1	387	3,140	40	(56)	156	551	1	(54)	0.4
	300 nmol/L	2	386	2,120	40	(56)	n.d.	n.d.	1	(54)	<i>N/A</i>
		3	377	1,280	100	(58)	170	141	40	(55)	4.2
PMO-14		1	387	1,900	40	(60)	169	108	40	(60)	5.4
	600 nmol/L	2	390	2,490	40	(60)	170	124	40	(60)	4.7
		3	385	1,680	60	(59)	175	157	40	(57)	5.9
		1	384	1,160	60	(59)	163	554	60	(59)	32.3
	10 μ mol/L	2	380	1,220	60	(59)	164	571	60	(59)	31.9
		3	385	2,220	40	(60)	165	923	40	(60)	29.4

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size	Conc.	Dilution	Fig.	Size	Conc.	Dilution	Fig.	

		(bp)	(pmol/L)	factor		(bp)	(pmol/L)	factor			
PMO-15	0 nmol/L	1	376	1,250	100	(64)	n.d.	n.d.	1	(61)	N/A
		2	390	1,720	60	(65)	n.d.	n.d.	1	(61)	N/A
		3	388	2,390	40	(63)	n.d.	n.d.	1	(61)	N/A
	300 nmol/L	1	384	1,820	40	(63)	n.d.	n.d.	1	(61)	N/A
		2	386	2,840	40	(63)	n.d.	n.d.	1	(61)	N/A
		3	384	2,090	40	(63)	150	572	1	(61)	0.7
	600 nmol/L	1	388	1,380	60	(65)	149	791	1	(61)	0.9
		2	391	3,230	40	(63)	150	601	1	(61)	0.5
		3	392	2,310	40	(63)	158	705	1	(62)	0.8
	10 μmol/L	1	391	1,680	40	(63)	169	462	40	(63)	21.6
		2	392	1,610	40	(63)	169	415	40	(63)	20.5
		3	388	1,910	40	(63)	169	504	40	(63)	20.9

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
PMO-16	0 nmol/L	1	381	2,050	40	(67)	n.d.	n.d.	1	(66)	N/A
		2	377	1,570	40	(67)	n.d.	n.d.	1	(66)	N/A
		3	379	1,630	40	(67)	n.d.	n.d.	1	(66)	N/A
	300 nmol/L	1	385	1,800	40	(68)	169	129	40	(68)	6.7
		2	383	1,550	40	(68)	167	94.4	40	(68)	5.7
		3	384	2,240	40	(68)	146	632	1	(66)	0.7
	600 nmol/L	1	391	2,130	40	(68)	170	267	40	(68)	11.1
		2	390	2,110	40	(68)	170	378	40	(68)	15.2
		3	389	2,220	40	(68)	170	282	40	(68)	11.3

	1	392	1,580	40	(68)	167	1,530	40	(68)	49.2
10 μ mol/L	2	386	3,660	40	(68)	171	2,890	40	(68)	44.1
	3	376	789	60	(69)	161	893	60	(69)	53.1

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Size (bp)	Skipped fragment		Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor			Conc. (pmol/L)	Dilution factor		
PMO-17	0 nmol/L	1	381	2,050	40	(71)	n.d.	n.d.	1	(70)	N/A
		2	377	1,570	40	(71)	n.d.	n.d.	1	(70)	N/A
		3	379	1,630	40	(71)	n.d.	n.d.	1	(70)	N/A
	300 nmol/L	1	378	1,910	40	(71)	n.d.	n.d.	1	(70)	N/A
		2	376	2,020	40	(71)	n.d.	n.d.	1	(70)	N/A
		3	374	2,200	40	(71)	n.d.	n.d.	1	(70)	N/A
	600 nmol/L	1	370	3,830	40	(71)	n.d.	n.d.	1	(70)	N/A
		2	365	3,100	40	(71)	n.d.	n.d.	1	(70)	N/A
		3	386	2,230	40	(72)	n.d.	n.d.	1	(70)	N/A
	10 μ mol/L	1	386	2,190	40	(72)	153	681	1	(70)	0.8
		2	387	2,650	40	(72)	152	545	1	(70)	0.5
		3	388	2,020	40	(72)	154	528	1	(70)	0.6

n.d.: not detected, *N/A*: Not Applicable

(2) Study No. 936-22-M-0661

176. The purpose of this study was to evaluate whether “weasel” antisense oligonucleotides comprising “at least 12 consecutive bases of” SEQ ID NO: 195 would induce exon 53 skipping. The UWA Patents define a “weasel” as “a cunningly designed antisense oligonucleotide” that is made “by joining together two or more antisense oligonucleotides

molecules.” See ’851 Patent at 4:56-61. The Court had previously construed the UWA Patent claims as encompassing “weasels,” but has since amended its construction. D.I. 248 at 10-11.

177. Again, a PMO targeting position +39+62 of exon 53 was selected as a positive control. A PMO targeting position +7+26 was selected as a negative control. PMOs-R1-R4 and R7 were designed as “weasels,” all of which contained at least 12 consecutive bases of SEQ ID NO: 195. PMOs-R5 and R6 were selected to evaluate the core “base sequences” 100% complementary to +31+42 and +36+47.

Study No. 936-22-M-0661				
Tested PMO	Length	Position on Exon 53	Skipping	Notes
#22 (PMO-22)	24 mer	+39+62	YES	Positive Control
#26 (PMO-26)	20 mer	+7+26	NO	Negative Control
#R1 (PMO-R1)	20 mer	+23+34; +81+88	NO	Experimental
#R2 (PMO-R2)	29 mer	+1+17; +36+47	YES	Experimental
#R3 (PMO-R3)	30 mer	+29+41; +159+175	NO	Experimental
#R4 (PMO-R4)	28 mer	+23+38; +47+58	YES	Experimental
#R5 (PMO-R5)	20 mer	Random 8 bases_+31+42	NO	Experimental
#R6 (PMO-R6)	30 mer	Random 13 bases_+36+47_Random_5 bases	NO	Experimental
#R7 (PMO-R7)	30 mer	+23+34; +128+145	NO	Experimental

178. As summarized above, despite each containing at least 12 consecutive bases of SEQ ID NO: 195, not all of the PMOs induced exon 53 skipping. For example, PMOs-R1, -R4 and -R7 each contain the same 12 consecutive bases of SEQ ID NO: 195 (i.e., +23+34), yet only PMO-R4 induced skipping. Similarly, PMOs-R2 and -R6 contain the same consecutive bases of SEQ ID NO: 195 (i.e., +36+47), yet only PMO-R2 induced skipping.

179. In my opinion, these data also supports my opinions above in a few ways. Again, because these experiments show varying levels of activity (including no activity in certain PMOs) when testing a variety of morpholino ASOs with thymine bases and different sets of “at least 12 consecutive bases of SEQ ID NO: 195,” they generally confirm the unpredictability of achieving exon skipping in exon 53 and that having 12 consecutive bases will not reliably induce exon skipping. Further, because each PMO tested was complementary to at least the seven base region (h53A(+36+42)) shared by all claimed ASOs of the '851 Patent, they contradict the notion that simply targeting that region with morpholino ASOs of 20-31 bases having thymine bases would reliably and predictably induce exon 53 skipping.

180. Additionally, because the weasels are 100% complementary to exon 53, these data also contradict the notion that 100% complementarity to exon 53 pre-mRNA and targeting the seven base region (h53A(+36+42)) shared by all claimed ASOs of the '851 Patent with morpholino ASOs having thymine bases would reliably and predictably induce exon 53 skipping. Likewise, the data suggest that consecutive complementary bases are not necessary to induce exon skipping (certain weasels did induced exon skipping). As such, these data are further evidence of the lack of structure-function relationship between exon 53-skipping and the structural features claimed in the UWA Patents.

181. A more detailed summary of the results obtained from this study is presented in the tables below.

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-22	0 nmol/L	1	378	2,560	20	(3)	n.d.	n.d.	1	(2)	N/A
		2	380	2,440	20	(3)	n.d.	n.d.	1	(2)	N/A

	3	380	2,790	20	(3)	n.d.	n.d.	1	(2)	N/A
	1	379	2,530	20	(3)	n.d.	n.d.	1	(2)	N/A
300 nmol/L	2	380	2,350	20	(3)	n.d.	n.d.	1	(2)	N/A
	3	378	2,680	20	(3)	n.d.	n.d.	1	(2)	N/A
	1	377	2,410	20	(3)	n.d.	n.d.	1	(2)	N/A
600 nmol/L	2	378	2,440	20	(3)	n.d.	n.d.	1	(2)	N/A
	3	379	2,060	20	(3)	n.d.	n.d.	1	(2)	N/A
	1	379	2,150	20	(3)	169	135	20	(3)	5.9
10 µmol/L	2	378	1,520	20	(3)	170	119	20	(3)	7.3
	3	377	1,780	20	(3)	168	165	20	(3)	8.5

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
		1	378	2,560	20	(6)	n.d.	n.d.	1	(4)	N/A
	0 nmol/L	2	380	2,440	20	(6)	n.d.	n.d.	1	(4)	N/A
		3	380	2,790	20	(6)	n.d.	n.d.	1	(4)	N/A
		1	380	3,210	20	(7)	n.d.	n.d.	1	(4)	N/A
	300 nmol/L	2	378	1,360	40	(8)	n.d.	n.d.	1	(4)	N/A
		3	379	2,330	20	(7)	n.d.	n.d.	1	(4)	N/A
PMO-26		1	377	2,730	20	(7)	n.d.	n.d.	1	(5)	N/A
	600 nmol/L	2	378	2,220	20	(7)	n.d.	n.d.	1	(5)	N/A
		3	377	2,850	20	(7)	n.d.	n.d.	1	(5)	N/A
		1	383	800	40	(8)	n.d.	n.d.	1	(5)	N/A
	10 µmol/L	2	382	2,340	20	(7)	n.d.	n.d.	1	(5)	N/A
		3	382	1,900	20	(7)	n.d.	n.d.	1	(5)	N/A

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-R1	0 nmol/L	1	381	2,620	20	(11)	n.d.	n.d.	1	(9)	<i>N/A</i>
		2	375	3,600	20	(11)	n.d.	n.d.	1	(9)	<i>N/A</i>
		3	378	2,360	20	(11)	n.d.	n.d.	1	(9)	<i>N/A</i>
	300 nmol/L	1	375	2,680	20	(11)	n.d.	n.d.	1	(9)	<i>N/A</i>
		2	375	2,740	20	(11)	n.d.	n.d.	1	(9)	<i>N/A</i>
		3	374	2,200	20	(11)	n.d.	n.d.	1	(9)	<i>N/A</i>
	600 nmol/L	1	381	1,470	20	(12)	n.d.	n.d.	1	(9)	<i>N/A</i>
		2	381	2,220	20	(12)	n.d.	n.d.	1	(9)	<i>N/A</i>
		3	383	2,430	20	(12)	n.d.	n.d.	1	(10)	<i>N/A</i>
	10 μ mol/L	1	381	1,920	20	(12)	n.d.	n.d.	1	(10)	<i>N/A</i>
		2	382	925	40	(13)	n.d.	n.d.	1	(10)	<i>N/A</i>
		3	378	3,940	20	(12)	n.d.	n.d.	1	(10)	<i>N/A</i>

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-R2	0 nmol/L	1	381	2,620	20	(17)	n.d.	n.d.	1	(14)	<i>N/A</i>
		2	375	3,600	20	(17)	n.d.	n.d.	1	(14)	<i>N/A</i>
		3	378	2,360	20	(17)	n.d.	n.d.	1	(14)	<i>N/A</i>
	300 nmol/L	1	375	1,850	20	(18)	n.d.	n.d.	1	(15)	<i>N/A</i>
		2	374	2,160	20	(18)	n.d.	n.d.	1	(15)	<i>N/A</i>
		3	372	2,300	20	(18)	n.d.	n.d.	1	(15)	<i>N/A</i>
	600 nmol/L	1	371	2,070	20	(18)	n.d.	n.d.	1	(15)	<i>N/A</i>

	2	372	1,810	20	(18)	n.d.	n.d.	1	(15)	N/A
	3	371	1,290	20	(18)	n.d.	n.d.	1	(15)	N/A
	1	368	1,230	20	(18)	168	164	5	(16)	3.2
10 μ mol/L	2	365	1,550	20	(18)	164	209	5	(16)	3.3
	3	384	1,070	20	(19)	164	237	5	(16)	5.2

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
		1	384	1,600	40	(21)	n.d.	n.d.	1	(20)	N/A
	0 nmol/L	2	383	1,650	40	(21)	n.d.	n.d.	1	(20)	N/A
		3	384	1,910	40	(21)	n.d.	n.d.	1	(20)	N/A
		1	382	1,710	40	(21)	n.d.	n.d.	1	(20)	N/A
	300 nmol/L	2	382	2,100	40	(21)	n.d.	n.d.	1	(20)	N/A
		3	381	2,580	40	(21)	n.d.	n.d.	1	(20)	N/A
PMO-R3		1	381	1,900	40	(21)	n.d.	n.d.	1	(20)	N/A
	600 nmol/L	2	383	1,990	40	(21)	n.d.	n.d.	1	(20)	N/A
		3	381	2,000	40	(21)	n.d.	n.d.	1	(20)	N/A
		1	383	1,830	40	(21)	n.d.	n.d.	1	(20)	N/A
	10 μ mol/L	2	381	1,800	40	(21)	n.d.	n.d.	1	(20)	N/A
		3	378	1,870	40	(21)	n.d.	n.d.	1	(20)	N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
		1	384	1,600	40	(24)	n.d.	n.d.	1	(22)	N/A
PMO-R4	0 nmol/L	2	383	1,650	40	(24)	n.d.	n.d.	1	(22)	N/A

	3	384	1,910	40	(24)	n.d.	n.d.	1	(22)	N/A
	1	387	1,900	40	(25)	n.d.	n.d.	1	(22)	N/A
300 nmol/L	2	388	2,100	40	(25)	n.d.	n.d.	1	(22)	N/A
	3	388	2,060	40	(25)	n.d.	n.d.	1	(22)	N/A
	1	388	1,660	40	(25)	n.d.	n.d.	1	(23)	N/A
600 nmol/L	2	387	3,020	40	(25)	n.d.	n.d.	1	(23)	N/A
	3	385	3,450	40	(25)	n.d.	n.d.	1	(23)	N/A
	1	374	1,160	40	(26)	167	127	40	(26)	9.9
10 µmol/L	2	371	1,110	40	(26)	167	155	40	(26)	12.3
	3	367	961	40	(26)	165	120	40	(26)	11.1

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
		1	384	965	60	(29)	n.d.	n.d.	1	(27)	N/A
	0 nmol/L	2	390	2,240	40	(30)	n.d.	n.d.	1	(27)	N/A
		3	388	3,830	40	(30)	n.d.	n.d.	1	(27)	N/A
		1	390	1,750	40	(30)	n.d.	n.d.	1	(27)	N/A
	300 nmol/L	2	383	3,350	40	(30)	n.d.	n.d.	1	(27)	N/A
		3	385	613	60	(29)	n.d.	n.d.	1	(27)	N/A
PMO-R5		1	376	3,200	40	(31)	n.d.	n.d.	1	(27)	N/A
	600 nmol/L	2	375	2,510	40	(31)	n.d.	n.d.	1	(27)	N/A
		3	374	3,300	40	(31)	n.d.	n.d.	1	(28)	N/A
		1	376	3,030	40	(31)	n.d.	n.d.	1	(28)	N/A
	10 µmol/L	2	377	1,090	40	(31)	n.d.	n.d.	1	(28)	N/A
		3	378	809	40	(31)	n.d.	n.d.	1	(28)	N/A

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-R6	0 nmol/L	1	384	965	60	(34)	n.d.	n.d.	1	(32)	<i>N/A</i>
		2	390	2,240	40	(35)	n.d.	n.d.	1	(32)	<i>N/A</i>
		3	388	3,830	40	(35)	n.d.	n.d.	1	(32)	<i>N/A</i>
	300 nmol/L	1	375	2,390	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
		2	376	1,890	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
		3	372	1,590	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
	600 nmol/L	1	372	1,710	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
		2	369	1,690	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
		3	369	1,860	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
	10 μ mol/L	1	370	1,600	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
		2	367	2,230	40	(36)	n.d.	n.d.	1	(33)	<i>N/A</i>
		3	376	2,170	40	(37)	n.d.	n.d.	1	(33)	<i>N/A</i>

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
PMO-R7	0 nmol/L	1	378	2,060	30	(39)	n.d.	n.d.	1	(38)	<i>N/A</i>
		2	381	3,440	30	(39)	n.d.	n.d.	1	(38)	<i>N/A</i>
		3	377	1,910	30	(39)	n.d.	n.d.	1	(38)	<i>N/A</i>
	300 nmol/L	1	375	3,290	30	(39)	n.d.	n.d.	1	(38)	<i>N/A</i>
		2	385	871	40	(40)	n.d.	n.d.	1	(38)	<i>N/A</i>
		3	374	2,930	30	(39)	n.d.	n.d.	1	(38)	<i>N/A</i>
	600 nmol/L	1	377	3,820	30	(39)	n.d.	n.d.	1	(38)	<i>N/A</i>

	2	376	4,020	30	(39)	n.d.	n.d.	1	(38)	N/A
	3	375	3,110	30	(39)	n.d.	n.d.	1	(38)	N/A
	1	377	3,770	30	(39)	n.d.	n.d.	1	(38)	N/A
10 μ mol/L	2	375	3,050	30	(39)	n.d.	n.d.	1	(38)	N/A
	3	378	1,800	40	(40)	n.d.	n.d.	1	(38)	N/A

n.d.: not detected, N/A: Not Applicable

182. Although the CERI reports were generated after the alleged June 2005 priority date of the UWA Patents, I understand that post-priority-date evidence can be considered where, as here, it is used to evaluate whether the disclosed species sufficiently represent the claimed genus. As shown above, many of the antisense oligonucleotides tested in the CERI reports that fell within the structural scope of the UWA Patents claims did not induce exon 53 skipping. The results of the CERI reports further support my opinion that a POSA, reading the specification of the UWA Patents, would not conclude that it sufficiently discloses the claimed genus of antisense oligonucleotides.

X. THE UWA PATENTS ARE NOT ENABLED

183. Practicing the full scope of the UWA Patent claims would require undue experimentation, particularly in view of their breadth, the unpredictability of the art, the lack of guidance and working examples provided in the UWA Patents, and the vast amount of time-consuming experimentation that would be required to test each and every member of the broadly claimed genus for exon 53 skipping activity.

A. The Breadth of the UWA Patent Claims

184. As discussed above, the scope of the UWA Patent claims is both structurally and functionally broad. The UWA Patent claims structurally encompass no fewer than tens of thousands of antisense oligonucleotides, and likely at least millions. Not only do the claims require

specific structural features, but they also require that the claimed antisense oligonucleotides induce exon 53 skipping. The claims, however, fail to specify a specific level of exon 53 skipping; thus they encompass a wide range of functional activity ranging from antisense oligonucleotides having “very faint skipping” activity to antisense oligonucleotides exhibiting stronger skipping that can induce a therapeutic effect. The UWA Patent claims thus encompass not only a structurally vast genus but also a functionally vast genus.

185. Of note, and as discussed in more detail above and below, simply because an antisense oligonucleotide has the claimed structural features does not mean that it necessarily will perform the required function of inducing exon 53 skipping. To the contrary, as discussed more fully by Dr. Wood in his reports and supplemental report, each of which I have reviewed in rendering my opinion, a POSA would need to screen each antisense oligonucleotide in order to determine if it induces exon 53 skipping as required by the UWA Patent claims. Thus, the number of potential antisense oligonucleotides that one would need to screen to determine whether that antisense oligonucleotide falls within the scope of the claim is vast.

B. The Nature of the Invention and the Unpredictability in the Art

186. As discussed by Dr. Wood and above, the ability of antisense oligonucleotides to induce dystrophin pre-mRNA exon skipping is unpredictable, even when using solely 100% complementary ASOs. Wood Report ¶¶ 75-89; *see also* Wood Interference Declaration ¶¶ 68-81; Wood Suppl. Rpt. ¶¶ 11-26. Indeed, as stated by the named inventors in the specification of the UWA Patents:

Attempts by the inventors to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense molecules compounds [sic] has been the result of empirical studies.

These empirical studies involved the use of computer programs to identify motifs potentially involved in the splicing process. Other computer programs were also used to identify regions of the pre-mRNA which may not have had extensive secondary structure and therefore potential sites for annealing of antisense molecules. Neither of these approaches proved completely reliable in designing antisense oligonucleotides for reliable and efficient induction of exon skipping.

'851 Patent at 32:15-30; Wilton Dep. Tr. at 54:4-13

By their own admission, the inventors of the UWA Patents could not use rational design or computer-based empirical studies to determine whether particular antisense oligonucleotides induced exon skipping. Thus, the inventors of the UWA Patents were unable to predict whether a particular antisense oligonucleotide could induce exon skipping based on sequence alone. Instead, each of the antisense oligonucleotides in the UWA Patents were individually tested using an *in vitro* assay to assess their exon 53 skipping activity. '851 Patent at 32:45-33:16. As shown in Table 39, several of the antisense oligonucleotides designed for exon 53 skipping using computer programs actually failed to induce skipping when individually tested (H53A(-12+10), H53A(-07+18), H53A(+07+26), and H53A(+124+145)).

187. Further, as discussed by Dr. Wood and above, even antisense oligonucleotides sharing a common sequence of differing lengths and 100% complementarity can exhibit a wide variety of exon skipping activity. Moreover, the UWA Patents themselves demonstrate that a change in only a single nucleotide can be dispositive of exon skipping activity. Wood Report ¶ 193 (H16A(-06+19) induced skipping at 25nM, H16A(-07+19) did not); *see also id.* ¶ 81 (deletion of as few as two nucleotides from an antisense oligonucleotide that induces exon skipping can reduce or eliminate such activity altogether); Wood Interference Declaration ¶ 74. Indeed, as named inventor Dr. Wilton himself admitted,

[REDACTED] Wilton Dep. Tr. at 185:23-186:9; WILTON0017692 at 694; WILTON0017925 at 927 ([REDACTED])

[REDACTED]; *see also* McClorey, Graham, et al. “Induced dystrophin exon skipping in human muscle explants.” *Neuromuscular Disorders* 16.9-10 (2006): 583-590 (“Moving an annealing site by only two or three bases can dramatically influence exon skipping efficacy of the AO (unpublished observations).”).

188. The UWA Patent claims not only require inducing exon 53 skipping, but they also require the antisense oligonucleotide be a morpholino antisense oligonucleotide. As Dr. Wood explains in his supplemental report, this reference to a “morpholino” antisense oligonucleotide not only encompasses PMOs that have six-membered morpholine rings in place of ribose with each nucleotide joined by phosphorodiamidate linkages, but it also encompasses antisense oligonucleotides using internucleotide linkages other than phosphodiamidate with the morpholine ring. Wood Suppl. Rpt. ¶¶ 82-85. As such, the UWA Patent claims requiring the antisense oligonucleotide be a “morpholino” antisense oligonucleotide encompasses more than just a PMO.

189. At the time of the invention in 2005, the use of morpholino antisense oligonucleotides in the field of DMD was in its infancy. Wood Suppl. Rpt. ¶¶ 29-52. As Dr. Wood explains in his supplemental report, as of 2005, the only commercial source of research quantities of morpholino oligonucleotides worldwide was AVI Biopharma and its spin-off Gene Tools. Wood Suppl. Rpt. ¶ 29. Gene Tools recommended antisense oligonucleotides that were 25 bases in length as their “longest available oligo,” while also noting that antisense oligonucleotides up to 30 bases in length could be available but for “a significant increase in price.” *Id.* Indeed, as Dr. Wilton testified, [REDACTED]

[REDACTED]

[REDACTED] Wilton Dep. Tr. at 205:4-13. Dr. Wood likewise explains how synthesizing morpholino ASOs generally was not within the ability of a POSA, and that no academic laboratory he was aware of had the capability and equipment to make morpholino ASOs themselves. Wood Suppl Rpt. ¶¶ 30-31. This comports with my own experience in the field at the time (and today).

190. As discussed above, historically, when a group of POSAs—whether an academic institution or company—decided to examine exon 53 skipping, those groups tested relatively few exon 53-directed ASOs. As of June 2005, the most exon 53 targeted ASOs any one had reported testing was Matsuo’s twelve ASOs. And *no one* had reported testing any exon 53 targeted morpholino ASO.

191. Of note, the scope of the UWA Patent claims is not limited to 25-mers, or even antisense oligonucleotides that are 30 bases or less, but instead encompasses antisense oligonucleotides that can be 20-31 bases in length.

192. Not only was it difficult, time-consuming, and expensive as of 2005 to obtain a morpholino antisense oligonucleotide longer than 25 bases in length, but as Dr. Wilton testified,

[REDACTED]

[REDACTED] Wilton Dep. Tr. at 163:8-164:18; *see also* Wood Suppl. Rpt. ¶ 22. Moreover, as of 2005, consistent techniques to assess dystrophin exon skipping both *in vitro* and *in vivo* had not yet been established. *Id.* ¶¶ 23-29. As such, not only would it be difficult and time-consuming to construct a morpholino antisense oligonucleotide as of 2005 but testing morpholino antisense oligonucleotides for exon 53 skipping would have required time-consuming and undue experimentation.

193. Notably, the UWA Patents provide no guidance to a POSA regarding how to overcome these known difficulties in the art as of 2005 for testing morpholino antisense oligonucleotides for exon 53 skipping. As I myself experienced when designing the CERI experiments, the specification of the UWA Patents description of experiments used to test antisense oligonucleotides for exon skipping would not have worked for morpholinos. In fact, I resorted to using the more modern Endo-Porter reagent, (which Dr. Wood indicates was not commercially available in June 2005, Wood Suppl. Rpt. ¶ 26) after an initial protocol designed to use contemporaneous methods of PMO delivery did not work. Thus, it is my conclusion that a POSA would have found it exceedingly difficult to determine exon skipping in PMOs as of 2005 based on the patent disclosures and known art.

194. Given the breadth of the claimed genus, as described above, testing the potential morpholino antisense oligonucleotides falling within the scope of the claims to determine if they induce exon 53 skipping would have required undue experimentation. As no structure-function relationship exists by which a POSA would have been able to determine whether a morpholino antisense oligonucleotide induces exon 53 skipping, a POSA would have been required to individually test all potential morpholino antisense oligonucleotides to determine if they fall within the scope of the claims and induce exon 53 skipping as required. Particularly since as of the June 28, 2005 priority date of the UWA Patents, only two morpholino antisense oligonucleotides had been tested for dystrophin exon skipping activity, Wood Suppl. Rpt. ¶¶ 39- 43, each potential morpholino antisense oligonucleotide would need to be tested to determine if it induces exon 53 skipping, which would have been a time-consuming and undue process.

195. Further illustrating the infancy of morpholino antisense oligonucleotides, as of 2005, there were only two reports on dystrophin exon skipping activity of morpholino antisense

oligonucleotides. Wood Suppl. Rpt. ¶¶ 39-43. As Dr. Wood explains in his report, and I incorporate by reference, one of these publications—GebSKI 2003—reported that morpholino antisense oligonucleotides should be at least 25 bases in length. *Id.* ¶ 43. But, as I have noted, the claims of the UWA Patents are broader, encompassing morpholino antisense oligonucleotides as small as 20 bases in length. I also note that this publication tested inducing exon 23 skipping, not inducing exon 53 skipping as the UWA Patent claims require. *Id.*

196. The other publication—Aartsma-Rus—targeted exon 46, not the claimed exon 53. Wood Suppl. Rpt. ¶ 41. The publication reported that the morpholino antisense oligonucleotides exhibited much lower exon skipping activity (5%) as compared to the 2'-O-Me-PS antisense oligonucleotide (75%). *Id.* As such, a POSA as of the time of the invention, particularly given the results in Aartsma-Rus, would not have expected that a 2'-O-MePS antisense oligonucleotide exhibiting exon skipping activity would have maintained such activity if it was converted to a morpholino backbone as required by the claims of the UWA Patents.

C. The Amount of Direction Provided by the Inventors and Working Examples

197. The UWA Patents also do not provide sufficient guidance or working examples to enable the broadly claimed genus of antisense oligonucleotides. As discussed above, the UWA Patents discuss using computer-based empirical studies for designing antisense oligonucleotides and the unreliability of using such studies to predict antisense oligonucleotides that induce exon skipping. '851 Patent at 32:15-30. Indeed, the inventors expressly acknowledged that these techniques failed to design antisense oligonucleotides that reliably induced exon 53 skipping. *Id.*

198. Despite the breadth of the claims, the UWA Patents only exemplify one pseudo-species that purportedly induces “very faint” exon 53 skipping and meets the “12 consecutive bases of SEQ ID NO: 195,” the “100% complementary to consecutive bases of a target region,” and the

“20 to 31 bases” limitations of the UWA Patent claims (i.e., SEQ ID NO: 195, also referred to as “H53A(+23+47)” in Table 39). SEQ ID NO: 195, however, is a 2'-O-methyl antisense oligonucleotide that includes uracil bases. SEQ ID NO: 195 does not include thymine bases nor is it a morpholino antisense oligonucleotide, which is a requirement of the UWA Patent claims. Thus, the UWA Patents provide no actual working examples showing antisense oligonucleotides encompassed by the UWA Patent claims that induce exon 53 skipping, let alone antisense oligonucleotides that have a therapeutic effect.

199. As described above, given that only two publications regarding dystrophin exon skipping activity of morpholino antisense oligonucleotides existed as of the priority date of the UWA Patents, and that one of those publications (Aartsma-Rus) reported significantly decreased activity when moving from a 2'-O-MePS antisense oligonucleotide to a morpholino antisense oligonucleotide, a POSA would not have expected that the data reported in the UWA Patents for SEQ 195 would have translated to an antisense oligonucleotide with a morpholino backbone. Particularly since SEQ 195 exhibited “very faint” exon 53 skipping with the 2'-O-MePS backbone, a POSA would not have reasonably expected it to necessarily maintain activity when converted to a morpholino.

200. Moreover, as Dr. Wood explains in his supplemental report, the requirement in the UWA Patent claims of having a “morpholino” antisense oligonucleotide not only encompasses PMOs that have six-membered morpholine rings in place of ribose with each nucleotide joined by phosphorodiamidate linkages, but it also encompasses antisense oligonucleotides using internucleotide linkages other than phosphodiamidate with the morpholine ring. Wood Suppl. Rpt. ¶¶ 82-85. Notably, the UWA Patents include no working examples of morpholino antisense oligonucleotides inducing exon 53 skipping.

201. Furthermore, the UWA Patents do not provide guidance for using SEQ ID NO: 195 as a starting point for the development of additional antisense oligonucleotides. In particular, the UWA Patents disclose:

Once efficient exon skipping had been induced with one antisense molecule, subsequent overlapping antisense molecules may be synthesized and then evaluated in the assay as described above. Our definition of an efficient antisense molecule is one that induces strong and sustained exon skipping at transfection concentrations in the order of 300 nM or less.

'851 Patent at 33:11-16.

202. As SEQ ID NO: 195 is reported to induce only very faint skipping at 50 nM, a POSA would not use SEQ ID NO: 195 to develop additional antisense oligonucleotides for exon 53 skipping based on the teachings of the UWA Patents. There simply is no teaching in the UWA Patents for selecting SEQ ID NO: 195 to develop additional antisense oligonucleotides for any purpose, let alone for therapeutic use. Because making and testing antisense oligonucleotides for exon skipping is a costly and labor-intensive process ([REDACTED]), a POSA would not select an antisense oligonucleotide, like SEQ ID NO: 195, that induced "very faint skipping at 50 nM" over SEQ ID NO: 193, which induced "strong skipping to 50 nM" for further optimization. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

D. The Quantity of Experimentation Required to Make and Use the Claimed Antisense Oligonucleotides

203. An enormous amount of experimentation would have been necessary to carry out the full scope of the claimed invention in 2005. Not only would it have taken a lot of work to make (e.g., synthesize) the full panoply of antisense oligonucleotides within the scope of the UWA Patent claims [REDACTED], the amount of screening required to practice the full scope of the claims would have been undue experimentation given the sheer breadth of antisense oligonucleotides that are potentially covered by the claims.

204. Even making and testing a small number of these antisense oligonucleotides would have required a massive investment of time, financial resources, and effort.³⁵ For example, in 2005, having a 30-base morpholino antisense oligonucleotide synthesized took typically two weeks, at least. The difficulty involved in synthesizing morpholino ASOs continued long past the date of the invention. A 2021 publication sponsored by Sarepta (and co-authored by Dr. Bradley L. Pentelute—who Sarepta retained to provide expert opinions regarding PMO synthesis in this litigation), for example, explained that “[c]hanges to the chemistry of PMO synthesis are greatly needed to enable the rapid drug development” because “with therapeutic PMO sequences on the order of 20 residues, synthesis times are on the order of weeks/” *See, e.g., Li et al., Fully automated fast-flow synthesis of antisense phosphorodiamidate morpholino oligomers*, Nature Comm’ns (2021). As the researchers explained, “[u]nsurprisingly, the production of screening libraries places **a significant burden** on the development of PMO drugs due to length protocols.” *Id.* (emphasis added).

³⁵ A single antisense oligonucleotide can cost U.S. \$200 to \$400 to have commercially synthesized.

205. The automated solution proposed (in 2021) by those researchers also demonstrates how time consuming morpholino synthesis was at the time of the invention (and remains). Li et al. report to “disclose an automated instrument that can expedite PMO synthesis by over an order of magnitude,” but their stated synthesis rate remains only “three therapeutic candidates for DMD in a single day.” *Id.* at 3. In other words, even with the improved technology sixteen years after the time of the invention, POSAs sponsored by Sarepta are still reporting that it would take more than three months to synthesize over 100 morpholino ASO candidates for DMD research.

206. Of course, the range of chemical modifications allowed by the claims only increases the amount of experimentation required. As an example, POSAs had successfully created chimeric morpholino ASOs in the years following June 2005.³⁶ But, even as late as 2023, POSAs recognized the continuing difficulties associated with morpholino-related synthesis. As Paul & Caruthers then explained, “limitations of th[e typical PMO] synthesis approach have been reported,” including that “the synthesis strategy for PMOs is not compatible with most other chemistries” one may use when making chimeric morpholino ASOs.³⁷ They therefore were experimenting with new ways to synthesize morpholino ASOs.

207. These publications exemplify why working with morpholino chemistry (which was then in its infancy) would substantially increase the amount of experimentation required to make and use the full scope of the genus. Making the “full scope” would require evaluating—at the specification’s urging—base modifications and chemical moieties with morpholino ASOs for which there was no guidance. To practice the full scope of the claims, a POSA would encounter

³⁶ Zhang et al., *Synthesis and properties of morpholino chimeric oligonucleotides*, Tetrahedron Letters 49:3570-3573 (2008).

³⁷ Paul & Caruthers, *Synthesis of Backbone-Modified Morpholino Oligonucleotides Using Phosphoramidite Chemistry*, Molecules, 28:5380 (2023).

(and be forced to resolve) difficulties such as these (*e.g.*, the slow synthesis, and synthesis incompatibilities), and do so without any meaningful guidance from the specification or the art on how to do so.

208. In vitro transfection and the subsequent RT-PCR study conservatively took several days. These methodologies at the time were relatively low throughput, only allowing a small number of antisense oligonucleotides to be screened at a time. Thus, testing a limited number of morpholino antisense oligonucleotide would have taken on the order of 2-3 weeks. Even making and testing a 2'-O-Me antisense oligonucleotides, which are typically easier and faster to synthesize, would have taken on the order of 3-5 days. And this is just to determine the *in vitro* efficacy using a screening procedure that does not examine production of dystrophin protein or whether a particular antisense oligonucleotide induces a therapeutic effect.

209. These time estimates for assaying, of course, assume that a POSA starts with both the morpholino ASOs and a reliable assay methodology. As Dr. Wood points out, however, reaching that point would itself be extraordinarily challenging for a POSA in June 2005. *See* Wood Suppl. Rpt. ¶¶ 22-59. Given the limited commercial availability of only certain PMOs at the time, the chemical synthesis efforts required to be able to make the full scope of the genus (*e.g.*, 31mers, ASOs with chemical moieties and nucleobase modifications) would itself be undue.

210. Because of the unpredictability of exon skipping, practicing the full scope of the claimed invention would have required synthesizing and testing each of these antisense oligonucleotides. This would have required tremendous and undue experimentation.³⁸

³⁸ The CERI Reports, which tested only 29 antisense oligonucleotides out of the tens of millions of antisense oligonucleotides encompassed by the genus of the UWA Patent claims, took upwards of 14 months to complete. *See* NS00102924 at 926, 990; NS00103061 at 063.

211. Indeed, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]³⁹

212. Moreover, [REDACTED]

[REDACTED]

[REDACTED] Wilton Dep.

Tr. at 174:12-17. Accordingly, in addition to selecting, synthesizing, and testing potential antisense

³⁹ Sarepta's Final Validity Contentions rely on the testimony of Mr. Satou, who is a named inventor of the NS patents, to argue that "companies like NS with more resources were capable of testing many antisense oligonucleotides at once in a short period of time." Sarepta Final Validity Contentions at 36. I understand that Mr. Satou was testifying as to the 2009-2010 timeframe (which I understand is the relevant timeframe for the NS patents), whereas the undue experimentation here is judged as of the June 28, 2005 priority date of the UWA Patents. I am not aware of high-throughput techniques for screening libraries of antisense oligonucleotides for exon skipping that were available in 2005.

oligonucleotides, one would also have to identify the correct assay conditions in order to make and use the invention claimed in the UWA Patents.

213. In essence, the UWA Patents disclose a starting point for further iterative research in an unpredictable field. Even synthesizing candidate antisense oligonucleotides would have required a tremendous quantity of experimentation. And testing the antisense oligonucleotides would then collectively have taken an immense amount of time and effort. Yet the UWA Patents offers very limited guidance in terms of representative species and no guidance in terms of a structure(s) common to all of the species within the claimed genus.

214. As a helpful point of comparison, the endeavor at-issue here—making and using the *full scope* of the claimed genus—would have been unprecedented. As discussed above and by Dr. Wood, *no one* at the time of the invention had tested more than twelve (12) exon 53-targeted ASOs at a single time or reported having tested any morpholino exon 53 ASOs. Wood Suppl. Rpt. ¶¶ 39-52; *supra* Section VIII.C. Even roughly six years later—after Sarepta and Nippon Shinyaku (pharmaceutical companies with greater resources than academic laboratories) had each filed patent applications describing exon 53 testing—there remained only one instance where 109 PPMOs (Sazani '586) were reported to have been tested and another where 66 PMOs (Poplewell 2009) were reported to have been tested. The most morpholino ASOs ever—even at that later time—tested by a group of POSAs for exon 53 was Poplewell's twenty-four (24).

215. Thus, exploring just the '851 Patent's 168 target regions would involve testing *seven (7) times more* morpholino exon 53-targeted ASOs than anyone had—even years after the date of the invention—ever tested. To suggest that such work (much less the many millions of ASOs actually encompassed by the claims) would be considered “routine experimentation” at the time borders on the absurd. Making and using the full scope of the genus would require a POSA

to conduct *far more* experimental work than the UWA researchers themselves report in the specification, and *far more* than any one had then performed.

216. To put it another way, if, in June 2005, a group of POSAs had either developed a reliable assay for morpholino ASOs or obtained data from testing a few dozen exon 53 PMOs (like Popplewell eventually did), that then-unprecedented work would have been worthy of publication in major journals. That amount and type of work would justify awarding a graduate student their Ph.D.

217. Accordingly, in my opinion making and using the full scope of the claims would require undue experimentation, and the UWA Patents are invalid for lack of enablement.

Exhibit 5

IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE

- - - - - x

NIPPON SHINYAKU CO., :
LTD., :
Plaintiff, : C.A. No. 21-1015 (JLH)
v. :
SAREPTA THERAPEUTICS, :
INC., :
Defendant. :

- - - - - x

SAREPTA THERAPEUTICS, :
INC. and THE :
UNIVERSITY OF WESTERN :
AUSTRALIA, Defendant :
and Counter-Plaintiff :
v. :
NIPPON SHINYAKU CO., :
LTD. and NS PHARMA, :
INC., Plaintiff and :
Counter-Defendants. :

DR. MATTHEW J.A. WOOD

Chicago, Illinois

Thursday, September 19, 2023

9:01 a.m.

Job No.: 545432

Pages: 1 - 132

Reported By: Cynthia J. Conforti

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	Q And a morpholino is also a structural	09:40:13
2	requirement, correct?	09:40:18
3	A It is.	09:40:18
4	Q You agree that the claim itself does not	09:40:19
5	say anything about using modified nucleobases,	09:40:21
6	correct?	09:40:29
7	A But it does not exclude them.	09:40:29
8	Q All right. And so just to get a clear	09:40:33
9	answer to my question, you agree that the claim	09:40:35
10	does not say anything about modified nucleobases?	09:40:39
11	A I agree, but because it -- they're not	09:40:44
12	specifically excluded, then one must interpret the	09:40:47
13	claim as including them.	09:40:49
14	Q And likewise, the claim does not recite	09:40:51
15	anything about being conjugated or attached in any	09:40:53
16	way to other moieties, correct?	09:40:59
17	A That's correct. But it doesn't	09:41:02
18	specifically exclude such moieties.	09:41:05
19	Q And then, finally, you agree that the	09:41:09
20	claim does not recite any internucleoside	09:41:12
21	linkages, correct?	09:41:19
22	A That's correct, but it does not exclude	09:41:20
23	variations on those types of linkages.	09:41:22
24	Q Well, you agree that the term "morpholino"	09:41:27
25	is used interchangeably with the term "PMO" which	09:41:33

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	does in fact fix particular internucleoside	09:41:40
2	linkages, correct?	09:41:43
3	MS. LO: Objection to form.	09:41:44
4	THE WITNESS: I said that they're used	09:41:46
5	interchangeably in a colloquial sense. In a	09:41:47
6	strictly and precise scientific sense, they're not	09:41:47
7	interchangeable. Morpholino refers to compounds	09:41:51
8	with a morpholino ring of which PMO is one type.	09:41:55
9	BY MR. FRAZIER:	09:42:17
10	Q Now, you're not offering opinions	09:42:17
11	concerning the validity of the '851 patent,	09:42:19
12	correct?	09:42:21
13	A That's correct.	09:42:21
14	Q You're not offering any opinions on the	09:42:22
15	written description requirement, correct?	09:42:25
16	A That's correct.	09:42:26
17	Q And you're not offering any opinions on	09:42:27
18	the enablement requirement?	09:42:29
19	A Correct.	09:42:31
20	Q So you have no view one way or another	09:42:33
21	that you're planning to offer in this case about	09:42:35
22	the validity of the '851 patent, correct?	09:42:38
23	A I haven't a view at all. I haven't -- I	09:42:41
24	haven't considered it.	09:42:44
25	Q You haven't considered the issue of the	09:42:45

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	validity of the '851 --	09:42:47
2	A No.	09:42:47
3	Q -- patent?	09:42:54
4	The answer is no, you have not?	09:42:54
5	A No, I have not.	09:42:55
6	Q And if you just give me a little bit more	09:42:58
7	time to finish my question before you start	09:43:00
8	talking, it will make the court reporter's life	09:43:02
9	much easier.	09:43:06
10	So in your view, are you indirectly	09:43:12
11	opining on the validity of the patent?	09:43:14
12	A No --	09:43:17
13	MS. LO: Objection to form.	09:43:18
14	THE WITNESS: -- I'm not -- I don't have a	09:43:19
15	direct or indirect opinion on validity of the	09:43:21
16	patent.	09:43:27
17	BY MR. FRAZIER:	09:43:33
18	Q You're not providing opinions to support	09:43:33
19	NS's arguments on invalidity but just not saying	09:43:37
20	the words "invalidity" yourself?	09:43:42
21	MS. LO: Objection, asked and answered.	09:43:43
22	THE WITNESS: I believe I've answered the	09:43:44
23	question.	09:43:46
24	BY MR. FRAZIER:	09:43:49
25	Q Okay. That's not a real objection from	09:43:49

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	your counsel. You have to go ahead and answer the	09:43:51
2	question. So it's not enough to just say "I think	09:43:54
3	I've answered my question."	09:43:56
4	A Well, I'm not expressing an opinion on the	09:43:57
5	validity of the patent.	09:44:01
6	Q Okay. Now, you promised in a sworn	09:44:08
7	declaration that was submitted to this court that	09:44:10
8	you would not be offering opinions concerning	09:44:13
9	validity of the '851 patent, correct?	09:44:16
10	MS. LO: Objection, mischaracterizes.	09:44:17
11	THE WITNESS: That's correct.	09:44:19
12	BY MR. FRAZIER:	09:44:21
13	Q And you gave that -- strike that.	09:44:21
14	You made that representation that you	09:44:28
15	would not be offering opinions on validity under	09:44:30
16	oath, correct?	09:44:32
17	A That's correct.	09:44:34
18	Q And you take that oath seriously, correct?	09:44:35
19	A I do.	09:44:38
20	Q And you're not going back on it now?	09:44:38
21	A No, I am not.	09:44:40
22	Q And you're aware that Dr. Hastings is	09:44:53
23	providing opinions concerning validity in this	09:44:56
24	case?	09:45:05
25	A Yes, I think I am.	09:45:05

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	Q And you have spoken to her?	09:45:06
2	A No, I have not spoken to Dr. Hastings.	09:45:08
3	Q Have you reviewed any of her reports?	09:45:11
4	A I've read one report.	09:45:16
5	Q Which report of Dr. Hastings have you	09:45:17
6	read?	09:45:22
7	A I think it was probably her most recent	09:45:22
8	report, which in fact I haven't thoroughly read,	09:45:25
9	but I've seen it.	09:45:28
10	Q And when did you see that report from	09:45:36
11	Dr. Hastings?	09:45:47
12	A I can't be certain, but it would have been	09:45:47
13	I guess probably within the last two or three	09:45:50
14	weeks.	09:45:52
15	Q As part of your preparation for this	09:45:52
16	deposition?	09:45:54
17	A Well, I received a copy of it. I've	09:45:54
18	looked at it very briefly. I haven't reviewed it	09:45:56
19	thoroughly.	09:45:59
20	Q Is it something that you considered in	09:46:00
21	connection with preparing your reply report, which	09:46:02
22	we have marked as Wood Exhibit 102?	09:46:05
23	A No.	09:46:10
24	Q Did you have Dr. Hastings' report in your	09:46:10
25	possession before September 4, 2024?	09:46:15

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	A	No.	09:48:41
2	Q	Were you disappointed by that?	09:48:42
3	MS. LO:	Objection.	09:48:46
4	THE WITNESS:	I'm not -- I have no opinion	09:48:46
5		on whether I'm disappointed on this.	09:48:47
6	BY MR. FRAZIER:		09:48:49
7	Q	But NS did call you to work on this case?	09:48:49
8	A	I was asked whether I could provide an	09:48:52
9		opinion on the state of the art in 2005 for exon	09:48:55
10		skipping.	09:48:59
11	Q	And you understood that Sarepta was on the	09:48:59
12		other side of the case?	09:49:02
13	A	I did.	09:49:03
14	Q	And you were okay with that?	09:49:04
15	A	I -- I don't really have an opinion as to	09:49:09
16		whether I was okay. I was asked to provide a	09:49:12
17		scientific opinion on the state of the art of exon	09:49:15
18		skipping, which I was happy to do.	09:49:18
19	Q	Dr. Wilton is a friend of yours?	09:49:21
20	A	I -- I know Dr. Wilton.	09:49:23
21	Q	Is he a friend of yours?	09:49:25
22	A	You know, if -- it depends how you define	09:49:27
23		the word "friend." I've known him for a long	09:49:29
24		time.	09:49:34
25	Q	NS offered to pay you for your work in	09:49:34

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	connection with this case, correct?	09:49:37
2	A Correct.	09:49:38
3	Q And you have been paid for your work in	09:49:39
4	this case, correct?	09:49:41
5	A That's correct.	09:49:41
6	Q More than \$10,000 by this point, correct?	09:49:42
7	A That's correct.	09:49:45
8	Q More than \$100,000 at this point?	09:49:46
9	A I -- I doubt it.	09:49:51
10	Q More than \$50,000?	09:49:52
11	A I think that's correct.	09:49:54
12	Q So between 50 and 100,000?	09:49:56
13	A I would have to check, but that would be	09:49:58
14	my guess.	09:50:01
15	Q Let's go back to Exhibit 101, which is the	09:50:33
16	opening supplemental expert report. And if you	09:50:37
17	turn to paragraph 58, you see there's a sentence.	09:50:46
18	It's at the bottom of paragraph 58 on page 21 that	09:51:11
19	begins: "Finally, that this publication..."	09:51:14
20	Do you see that?	09:51:18
21	A Yes.	09:51:18
22	Q And it says:	09:51:18
23	Finally, that this publication came two	09:51:19
24	years after the priority date reflects that	09:51:22
25	Drs. Wilton and Fletcher had neither made nor	09:51:26

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	recognized nor appreciated that the reported	09:51:30
2	exon-skipping activity of SEQ ID NO: 195 or other	09:51:32
3	"sub-optimal" sequences could be extrapolated to	09:51:37
4	the PMO backbone chemistry as of the priority	09:51:40
5	date.	09:51:44
6	Do you see that?	09:51:45
7	A I do.	09:51:46
8	Q All right. And that terminology, "neither	09:51:46
9	recognized nor appreciated," is that scientific	09:51:50
10	terminology you use?	09:51:57
11	A It was -- it's certainly partially	09:52:02
12	scientific.	09:52:04
13	Q So if I were to look at your other	09:52:06
14	scientific publications, I would see a phrase like	09:52:09
15	"neither recognized nor appreciated a particular	09:52:13
16	result"?	09:52:16
17	A You may.	09:52:17
18	Q Or more likely it's legal terminology that	09:52:19
19	was drafted for this case, correct?	09:52:23
20	A That's -- that's also true, yes.	09:52:26
21	Q And same thing, if you turn to the	09:52:46
22	beginning of Section 7 on the next page, page 22,	09:52:48
23	you see the heading A says:	09:52:54
24	Post-Priority Date Evidence Reflects a	09:52:57
25	Lack of Recognition or Appreciation of the Claimed	09:53:01

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	Genus by Wilton and Others?	09:53:05
2	A Yes.	09:53:06
3	Q All right. And, again, that recognition	09:53:07
4	or appreciation, that's legal terminology,	09:53:10
5	correct?	09:53:12
6	A It's certainly legal terminology, yes.	09:53:13
7	Q And if you turn to paragraph 76. You see	09:53:36
8	at the bottom of paragraph 76 it says:	09:54:01
9	The inventors of the UWA Patents did not	09:54:03
10	recognize or appreciate the parameters of the	09:54:05
11	claimed genus when the UWA patents were filed on	09:54:08
12	June 28, 2005.	09:54:11
13	Do you see that?	09:54:12
14	A Yes.	09:54:13
15	Q All right. And same thing, that that's --	09:54:13
16	that "did not recognize or appreciate" is legal	09:54:16
17	terminology that you're using, correct?	09:54:18
18	A It's also scientific.	09:54:21
19	Q But it's a legal phrase that you use for	09:54:25
20	purposes of your analysis in this case, not	09:54:26
21	something that you typically use in your	09:54:28
22	scientific publications, correct?	09:54:30
23	A I would agree with that, yes.	09:54:32
24	Q So one of the issues that you commented on	09:55:27
25	in connection with your reports, Exhibits 101 and	09:55:29

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	102, was delivery of antisense oligonucleotides	09:55:37
2	for cells, correct?	09:55:41
3	A Correct.	09:55:42
4	Q And you agree that in the case of PMOs,	09:55:42
5	there were strategies that were known in the art	09:55:49
6	for delivering PMOs to cells in the laboratory	09:55:53
7	in vitro, correct?	09:55:59
8	MS. LO: Objection, vague.	09:56:01
9	THE WITNESS: As of 2005?	09:56:05
10	BY MR. FRAZIER:	09:56:05
11	Q Yes.	09:56:06
12	A There were one or at most two reports of	09:56:10
13	possible methods that could be used that weren't	09:56:15
14	well established.	09:56:18
15	Q One of those is a report by Gebski in	09:56:22
16	2003, correct?	09:56:28
17	A That's correct.	09:56:29
18	Q And that report used a methodology called	09:56:31
19	"leashes," correct?	09:56:35
20	A That's correct.	09:56:36
21	Q And that report showed that they could	09:56:40
22	enhance delivery of PMO antisense oligonucleotides	09:56:44
23	using the leashes as a strategy, correct?	09:56:51
24	MS. LO: Objection to form.	09:56:54
25	THE WITNESS: Well, that's true, but a	09:56:56

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	MS. LO: Objection, outside the scope.	12:34:10
2	THE WITNESS: That's not necessarily my	12:34:12
3	understanding.	12:34:13
4	BY MR. FRAZIER:	12:34:15
5	Q Why would that be?	12:34:15
6	A Well, because they -- they would compete	12:34:16
7	with any therapy that is developed for muscular	12:34:18
8	dystrophy, including NS, Sarepta, and a whole host	12:34:22
9	of other companies.	12:34:26
10	Q To your knowledge, has PepGen made any	12:34:40
11	overtures to NS with respect to its product for	12:34:45
12	the treatment of Duchenne's muscular dystrophy?	12:34:51
13	A No, not to my knowledge.	12:35:01
14	MR. FRAZIER: Why don't we take a short	12:35:18
15	break, and I'll see if I can wrap everything else	12:35:19
16	into a small module and we'll wrap up.	12:35:22
17	THE VIDEOGRAPHER: Stand by. We are going	12:35:27
18	off the record. The time is 12:34 p.m.	12:35:29
19	(Recess taken).	12:47:29
20	THE VIDEOGRAPHER: We are going back on	12:47:29
21	the record. The time is 12:46 p.m.	12:47:31
22	MR. FRAZIER: Let's mark as Wood Exhibit	12:47:34
23	116 a document Bates number NS143268 through 451.	12:47:37
24	(Wood Exhibit 116 marked for	12:48:06
25	identification.)	12:48:07

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	BY MR. FRAZIER:	12:48:07
2	Q You recognize Exhibit 116, Dr. Wood?	12:48:08
3	A Yes, I do.	12:48:30
4	Q And this is a declaration that you	12:48:32
5	prepared in connection with the interference	12:48:34
6	proceeding that you were involved with on behalf	12:48:37
7	of Sarepta?	12:48:41
8	A Yes.	12:48:41
9	Q All right. And if you turn to	12:48:42
10	page 143422.	12:48:46
11	A Yes.	12:49:00
12	Q And this is your signature here?	12:49:00
13	A It is.	12:49:02
14	Q And so does this, then, refresh your	12:49:02
15	recollection that you prepared this in late 2014,	12:49:06
16	around November of 2014?	12:49:10
17	A Yes.	12:49:11
18	Q Okay. And among the topics that you	12:49:13
19	covered in this declaration was the chemistries	12:49:21
20	for antisense oligonucleotides, correct?	12:49:27
21	A That would be correct, yes.	12:49:31
22	Q And if you turn to paragraph 52.	12:49:32
23	A Yes.	12:49:46
24	Q You see in paragraph 52, you're talking	12:49:47
25	about various types of antisense oligonucleotide	12:49:51

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	chemistries that are different from	12:49:57
2	naturally-occurring nucleotides, correct?	12:50:01
3	A Yes.	12:50:03
4	Q And you say at the bottom of paragraph 52:	12:50:04
5	These include phosphorothioates, which you	12:50:06
6	abbreviate PS, morpholinos, which you abbreviate	12:50:13
7	PMOs, and then two other types, right?	12:50:20
8	A Correct.	12:50:21
9	Q And so it -- and then you provide a	12:50:22
10	picture of a morpholino, which you put in parens	12:50:25
11	PMO, on the right side of the figure below,	12:50:32
12	correct?	12:50:35
13	A Correct.	12:50:35
14	Q So in presenting this information to the	12:50:36
15	Patent Office on behalf of Sarepta, you described	12:50:40
16	morpholinos as being phosphorodiamidate linkages,	12:50:45
17	correct?	12:50:53
18	MS. LO: Objection to form.	12:50:54
19	THE WITNESS: Well, I think paragraph 55	12:50:58
20	says that phosphorodiamidate and morpholino	12:51:06
21	oligomers are called morpholinos colloquially.	12:51:13
22	BY MR. FRAZIER:	12:51:16
23	Q Well, it doesn't say colloquially. It	12:51:16
24	says phosphorodiamidate morpholino oligomers	12:51:19
25	called morpholinos or PMOs?	12:51:23

Transcript of Dr. Matthew J.A. Wood
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1	That's what you said, right?	12:51:24
2	A Correct.	12:51:26
3	Q You stand beside -- behind this	12:51:27
4	declaration from ten years ago?	12:51:31
5	MS. LO: Objection, vague.	12:51:32
6	THE WITNESS: Well, I do.	12:51:35
7	MR. FRAZIER: Let me mark as Exhibit 117 a	12:51:48
8	copy of a declaration of Michelle L. Hastings	12:52:00
9	dated March 20, 2023.	12:52:05
10	MS. LO: I'll object to this exhibit as	12:52:11
11	outside the scope of Dr. Woods' expert reports.	12:52:13
12	MR. FRAZIER: Try to link that up.	12:52:17
13	(Wood Exhibit 117 marked for	12:52:28
14	identification.)	12:52:49
15	BY MR. FRAZIER:	12:52:49
16	Q Do you recognize 117? Ever seen this	12:52:50
17	before?	12:52:52
18	A I don't believe it's cited in my reports.	12:52:52
19	Q And you see it's a declaration of	12:52:58
20	Michelle L. Hastings from 2023, and you understand	12:52:59
21	that Dr. Hastings is providing opinions on behalf	12:53:04
22	of NS in this case?	12:53:07
23	A I do.	12:53:08
24	Q And this is -- this is a declaration that	12:53:09
25	she provided in connection with claim construction	12:53:15

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	proceedings in this case. Just as background.	12:53:18
2	And if turn to pages 39 and 40, and you	12:53:27
3	see that she has paragraph 111 that refers to a	12:53:37
4	review article by Summerton and Weller from 1997,	12:53:44
5	describes the design and preparation of	12:53:48
6	morpholinos, and notes the structure of morpholino	12:53:50
7	oligomers includes uracil bases.	12:53:56
8	Do you see that?	12:53:59
9	A Yes.	12:54:00
10	Q And then she includes the illustration	12:54:00
11	with the subtitle Morpholino Oligo Structure below	12:54:04
12	it, right?	12:54:09
13	A Correct.	12:54:09
14	Q So you understand that to be informing the	12:54:10
15	Court that the structure of a morpholino oligo has	12:54:17
16	a phosphoramidate linkage, correct?	12:54:21
17	MS. LO: I'm going to object as outside	12:54:24
18	the scope. This document is -- I don't think	12:54:26
19	Dr. Wood has seen this document before, and it's	12:54:29
20	Dr. Hastings' declaration on which you have	12:54:32
21	already questioned her.	12:54:37
22	BY MR. FRAZIER:	12:54:39
23	Q You may proceed.	12:54:39
24	MS. LO: I also object to the form of the	12:54:40
25	question.	12:54:42

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	Q Let's take a look at that.	12:57:49
2	MR. FRAZIER: We'll mark as Exhibit 118 a	12:57:58
3	1997 publication by Summerton and Weller.	12:58:03
4	(Wood Exhibit 118 marked for	12:58:23
5	identification.)	12:58:40
6	BY MR. FRAZIER:	12:58:40
7	Q And is this the article by Summerton and	12:58:40
8	Weller you're referring to?	12:58:43
9	A Yes, I believe it is.	12:58:44
10	Q And if you look at the section on page 189	12:58:52
11	under the heading Intersubunit Linkage?	12:58:54
12	A Yes.	12:59:00
13	Q Is that the part of the article you're	12:59:01
14	relying on for your view that in 2005 a person of	12:59:05
15	skill in the art would have thought that any	12:59:10
16	intersubunit linkage was appropriate for an	12:59:16
17	antisense oligonucleotide for exon skipping?	12:59:17
18	MS. LO: Objection to form.	12:59:21
19	THE WITNESS: Well, it's certainly one of	12:59:24
20	the parts of the publication. I mean, this as a	12:59:26
21	whole talks about a broad range of morpholinos,	12:59:32
22	how to design them, prepare them, their	12:59:35
23	properties, including the internucleotide	12:59:37
24	linkages. That could be used, as the abstract	12:59:43
25	says, to treat a broad range of intractable	12:59:46

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	diseases.	12:59:49
2	BY MR. FRAZIER:	12:59:49
3	Q Right. And it says:	12:59:50
4	Although morpholino oligos containing a	12:59:50
5	number of such linkages provide effective binding	12:59:54
6	to target genetic sequences, consideration of cost	12:59:58
7	and ease of synthesis, chemical stability, aqueous	13:00:01
8	solubility, and affinity and homogeneity of	13:00:04
9	binding to RNA led us to focus on	13:00:06
10	phosphorodiamidate shown in Figure 2, right?	13:00:10
11	A Correct.	13:00:12
12	Q So that was something that the field was	13:00:12
13	well aware of, that there were all those drawbacks	13:00:14
14	to the other types of linkages, correct?	13:00:16
15	MS. LO: Objection to form.	13:00:18
16	THE WITNESS: Well, there were other types	13:00:23
17	of linkages that could be used, and this was of	13:00:25
18	course 1997.	13:00:28
19	BY MR. FRAZIER:	13:00:33
20	Q And in the time frame between 1997 and	13:00:33
21	2005, had you seen adoption of any other types of	13:00:37
22	linkages in the area of antisense oligonucleotides	13:00:39
23	for exon skipping?	13:00:45
24	A Well, as I said previously, only two	13:00:46
25	morpholinos had been used for exon skipping by	13:00:49

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

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1	2005, two -- only two molecules.	13:00:52
2	Q All right. So, no, you had not seen	13:00:55
3	others using other internucleotide linkages,	13:00:57
4	correct?	13:01:00
5	A But they hadn't been tested. They could	13:01:00
6	have been used.	13:01:03
7	Q You said they could have been used?	13:01:04
8	A Well, this makes it very clear that the	13:01:06
9	methods for their preparation were possible.	13:01:09
10	Q You don't read this as recommending	13:01:13
11	against using it?	13:01:15
12	A No.	13:01:21
13	MR. FRAZIER: I have nothing further at	13:01:59
14	this time.	13:02:02
15	MS. LO: Can I have just a few minutes?	13:02:02
16	THE VIDEOGRAPHER: Stand by. We are going	13:02:08
17	off the record. The time is 1:01 p.m.	13:02:10
18	(Recess taken.)	13:03:20
19	THE VIDEOGRAPHER: We are going back on	13:03:27
20	the record. The time is 1:02 p.m.	13:03:29
21	EXAMINATION	13:03:31
22	BY MS. LO:	13:03:31
23	Q Dr. Wood, if I can direct your attention	13:03:32
24	to paragraph 76 of your supplemental expert	13:03:34
25	report, Exhibit 101.	13:03:38

Transcript of Dr. Matthew J.A. Wood
Conducted on September 19, 2024

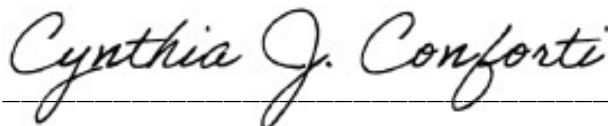
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1 CERTIFICATE OF COURT REPORTER - NOTARY PUBLIC

2
3 I, Cynthia J. Conforti, Certified
4 Shorthand Reporter No. 084-003064, CSR, CRR, and a
5 Notary Public in and for the County of Cook, State
6 of Illinois, the officer before whom the
7 foregoing deposition was taken, do hereby certify
8 that the foregoing transcript is a true and
9 correct record of the testimony given; that said
10 testimony was taken by me stenographically and
11 thereafter reduced to typewriting under my
12 direction; that reading and signing was requested;
13 and that I am neither counsel for, related to, nor
14 employed by any of the parties to this case and
15 have no interest, financial or otherwise, in its
16 outcome.

17 IN WITNESS WHEREOF, I have hereunto set my
18 hand and affixed my notarial seal this 23rd day of
19 September, 2024.

20
21 My commission expires: January 4, 2028

22 
23 _____

24 Notary Public in and for the
25 State of Illinois

Exhibit 6

IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE

NIPPON SHINYAKU CO., LTD.,)

Plaintiff,)

v.)

SAREPTA THERAPEUTICS, INC.,)

Defendant.)

C.A. No. 21-1015 (JLH)

SAREPTA THERAPEUTICS, INC. and)
THE UNIVERSITY OF WESTERN)
AUSTRALIA, Defendant and Counter-)
Plaintiff)

v.)

NIPPON SHINYAKU CO., LTD. and)
NS PHARMA, INC., Plaintiff and)
Counter-Defendants.)

SUPPLEMENTAL REPLY EXPERT REPORT OF DR. MATTHEW J.A. WOOD



September 4, 2024

Matthew J.A. Wood, F.Med.Sci., MA, D.Phil.

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I. INTRODUCTION

1. As set forth in my July 3, 2024 Supplemental Report (“Wood Suppl.”), I have been asked by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) to provide supplemental expert opinions concerning U.S. Patent No. 9,994,851 (“the ’851 patent”) and two other asserted University of Western Australia (“UWA”) patents, U.S. 10,227,590 and U.S. 10,266,827 (together with the ’851 patent, the “UWA Patents”) in view of the Court’s clarification and/or amendment of its prior claim construction order. I submit this report in reply to the August 14, 2024 Supplemental Rebuttal Expert Report of Steven F. Dowdy, Ph.D. (“Dowdy Suppl. Reb.”).

II. QUALIFICATIONS, MATERIALS CONSIDERED, AND LEGAL STANDARDS

2. A discussion of my qualifications and compensation was provided in my Opening Report. The opinions I express in this report are based on my knowledge and experience, and on the numerous documents I have reviewed and considered to date. A list of materials I have considered in connection with this reply report is attached as Exhibit 1.

3. I understand that, from the claims of the UWA Patents, Sarepta is now only asserting claim 1 of the ’851 patent against NS, therefore I have focused my analysis on that claim. However, my opinions are equally applicable to the other claims of the UWA Patents because they share a common specification, and the claims include many identical requirements. Because Dr. Dowdy addresses all three of the UWA Patents in his Supplemental Rebuttal Report, I address them collectively here.

4. I have been informed by counsel that the parties have agreed that a person of ordinary skill in the art (“POSA”) for the UWA Patents is an individual that has an M.D., Ph.D. or lower degree with expertise in molecular biology, biochemistry or a related area, and experience with neuromuscular or genetic diseases and/or designing and testing antisense oligonucleotides for splice-

site switching/exon skipping applications. The POSA would have general knowledge of antisense oligonucleotide chemical modifications to the backbone, nucleobases and other manipulations that can alter the activity of the antisense molecule, as well as delivery methods for antisense oligonucleotides. A POSA would also have general knowledge regarding using antisense oligonucleotides in cell-free, cell-based and/or in vivo experiments, as well as DMD models and the use of antisense oligonucleotides to induce skipping of DMD exons to correct the open reading frame of the RNA transcripts. I was a POSA as of 2005. I have rendered my opinions from the perspective of a POSA.

III. REPLY TO DR. DOWDY'S OPINIONS

5. As a preliminary matter, I disagree that the arguments in my Supplemental Report are not responsive to the Court's clarified construction. *Cf.* Dowdy Suppl. Reb. ¶ 170. My opinions regarding unpredictability expressly take into account the new 100% complementarity requirement. *See, e.g.,* Wood Suppl. ¶ 12. The same is true for my opinions concerning morpholino antisense oligonucleotides ("AONs") and the scope of the UWA Patents' claims. *See, e.g.,* Wood Suppl. ¶¶ 53-54, 62.

A. Dr. Dowdy Was Not Working in the Field in June 2005

6. Dr. Dowdy renders many opinions regarding what a POSA would have known, would have expected, would have been able to do, and would (or would not) have understood. *See, e.g.,* Dowdy Suppl. Reb. at ¶¶ 13, 18, 22, 28, 46, 53 etc. To my recollection, Dr. Dowdy was not working in the field of exon skipping AONs in June 2005.¹ Nor was Dr. Dowdy studying DMD in June 2005. As stated in my October 11, 2023 Rebuttal Report ("Wood Reb."), there were only a few groups

¹ Dr. Dowdy characterized the field of the UWA Patents as "exon skipping ASOs." *See, e.g.,* Dowdy Reb. ¶ 6.

working to develop exon skipping AONs to treat DMD in 2005. Wood Reb. ¶ 12. Dr. Dowdy did not belong to any of them.

7. Based on my review of Dr. Dowdy's curriculum vitae (DTX-1334), it appears that he was working on protein or peptide therapeutics in the 2005 time frame. His first publication involving any sort of antisense technology was in 2007, and related to siRNA delivery (Meade & Dowdy, Exogenous siRNA delivery using peptide transduction domains/cell penetrating peptides. *Adv. Drug Deliv Rev.* 59:134-140 (2007). Double-stranded siRNA is not only structurally different from single-stranded AONs for exon skipping, it operates through a vastly different mechanism. siRNA facilitates gene silencing (RNA interference) through recruitment of Ago2 in the RNAi pathway. The target mRNA (not pre-mRNA) is degraded as a primarily cytoplasmic activity. This is quite distinct from the RNase H antisense mechanism and very distinct from exon skipping. Therefore, I do not consider Dr. Dowdy's siRNA research to fall within the exon skipping field.

8. Dr. Dowdy's lack of research experience in the field and technology at the relevant time frame has affected the accuracy of his opinions. For example, Dr. Dowdy claims that "by June 2005, researchers also had access to patient-derived cells and had extensive experience with growing and testing them." Dowdy Suppl. Reb. ¶ 194. As Dr. Dowdy would know if he had been working in the field of DMD research in 2005, there was very limited access to patient cells. Before proof of concept of exon skipping by in the mid-1990s by Dr. Matsuo's group in Japan,² there was little reason to obtain, characterize, catalog, and store samples from DMD patient biopsies. As of 2005, very few hospitals had the permissions and infrastructure in place to do so, and even local hospital researchers had limited access to a small number of patient samples. Based on my literature search, there were only two such sources: Leiden University (where Drs. Aartsma-Rus, van Deutekom, van Ommen,

² Pramano et al., *Biochem Biophys Res Commun* 1996; 226:445-449.

and others worked), and Kobe University in Japan (where Dr. Matsuo worked). *See* McClorey et al., *Curr Op Pharmacol* 2005; 5:529-534 (“McClorey 2005”) at 531 (citing references from those two groups). Thus, the van Ommen and Matsuo groups that Dr. Dowdy cites as examples were the sole exceptions rather than the rule. For example, Dr. Wilton’s group did not publish studies using any patient cells until 2009. Mitrpant et al., *Mol Ther.* Aug. 2009; 17(8):1418-26 (“Mitrpant 2009”) (describing assays with patient cells having a mutation in exon 16). In sum, as of June 2005, no biobanks for patient cells as we currently know them existed. There was no routine access to genetically well-characterized patient cells, or genetically diverse patient cells encompassing the full range of DMD mutations or even a limited range relevant to skipping a single exon.³ Dr. Dowdy’s claim that DMD patient cells were readily available to researchers (Dowdy Suppl. Reb. ¶ 194) is factually inaccurate.

9. In another example, Dr. Dowdy’s opinions that a POSA would regard any level of exon skipping as “a meaningful achievement, worth further pursuit and optimization” (Dowdy Suppl. Reb. ¶ 107) and that a POSA would pay no heed to the level of skipping or experimental condition or assay used (*id.* ¶¶ 107, 149) are also inconsistent with the approach of researchers working in the field in 2005. Then, as now, the goal was to identify effective AONs and develop them into clinical candidates and then therapeutics. *See, e.g.,* Aartsma-Rus et al., *Gene Ther* 2004; 11:1391-98 (“Aartsma-Rus 2004”) at 1393 (“For future clinical studies, the preferred AON analog induces the highest levels of exon skipping at low levels of cytotoxicity.”). The UWA Patents contemplate this goal. *See, e.g.,* ’851 patent, Col. 28:39-43 (“in one embodiment the present invention provides

³ Aartsma-Rus et al., *Hum. Mol. Genet.* 2003; 12(8):907-914 reports one patient cell sample suitable for exon 53 skipping.

antisense molecules that bind to a selected target in the dystrophin pre-mRNA to induce efficient and consistent exon skipping described herein in a therapeutically effective amount”).

10. A minimal level of exon 53 skipping would not meet this goal, and a POSA would not have pursued or attempted to optimize an AON that exhibited a minimal level of skipping in the *in vitro* assays described in the ’851 patent. *See* Aung-Htut et al, Int. J. Mol. Sci. 2019; 20:5030 (“Aung-Htut 2019b”) at 6 (“when developing any AO for clinical use, it is obvious that the most appropriate compound will be one that induces robust splice switching at a low concentration”). Nor was achieving any level of exon 53 skipping a “meaningful achievement” as of June 2005. *Cf.* Dowdy Suppl. Reb. ¶ 107. Two groups had already published such results. Wood Rebuttal ¶¶ 13 (discussing Aartsma-Rus et al., Neuromuscular Disorders 2002; 12:S71-77 and patent publications from Dr. Matsuo’s group in Japan).

11. Dr. Dowdy’s lack of experience in the exon skipping field is also reflected in the divergence between what he says a POSA could have or would have done, and what researchers working in the field actually did. As discussed in my Supplemental Report and further below, the real-world evidence regarding whether researchers (1) made their own PMOs; (2) were capable of testing hundreds of PMOs in a matter of months; or (3) recognized that the UWA Patents disclosed the claimed genus of exon 53 skipping AONs uniformly contradicts Dr. Dowdy’s opinions.

B. The ‘007 Interference

12. Dr. Dowdy offers opinions regarding the “context” of Interference Proceeding No. 106,007 before the Patent Trial and Appeal Board (“the ‘007 Interference”). Dowdy Suppl. Reb. ¶¶ 84-89. He claims that UWA and Sarepta’s statements were “about generalized unpredictability” and “not relevant” to the claims of the UWA Patents. *Id.* ¶ 89. I served as an expert witness on behalf of UWA and Sarepta in the ‘007 Interference, which was a dispute between UWA and Academisch Ziekenhuis Leiden (“AZL”). The ‘007 Interference involved an AZL patent and UWA’s U.S. Patent

No. 8,455,636, which claims priority to the same 2005 PCT application as the UWA Patents at issue in this litigation.

13. The opinions I offered in the ‘007 Interference regarding the unpredictability of exon skipping are equally applicable to the field and timeframe at issue here. My Opening Report in this matter was largely drawn from the Technical Background section of my November 18, 2014 declaration submitted on behalf of UWA in the ‘007 Interference (“Wood ‘007 Decl.”). Opening Report ¶ 17. I also offered a second declaration in the ‘007 Interference (“Wood 2nd ‘007 Decl.”).

14. Other statements from my ‘007 Interference Declarations are also applicable in this litigation. For example:

- “Given the unpredictability of this technology (see, for example, paragraphs 68-81), a person of ordinary skill in the art could not predict from h53AON1 the operability of any other AONs having nucleotide sequences that differ from h53AON1.” Wood ‘007 Decl. ¶ 280;
- “In my opinion... using AONs to induce exon skipping was a very unpredictable technology in March of 2003, and it remains so to this day.” *Id.* ¶ 282;
- “This recognition of the lack of predictability continued beyond 2005. A 2007 paper co-authored by several members of the AZL group states that ‘several years after the first attempts at dystrophin exon skipping with AOs [antisense oligonucleotides], ***there are still no clear rules to guide investigators in their design***, and in mouse and human muscle cells *in vitro* ***there is great variability for different targets and exons.***’; emphasis added.” *Id.* ¶ 291 (quoting Archevala-Gomez 2007 at 807);
- “And again in 2009 the AZL investigators wrote that while existing software programs can facilitate design, ‘in general a trial and error procedure is still involved to identify potent AONs.’” *Id.* ¶ 292 (quoting Aartsma-Rus 2009);
- “studies performed before, and long after, the filing date of the AZL applications demonstrated that small changes in nucleotide sequences in overlapping AONs can convert an AON from one that induces skipping to one that does not.” *Id.* ¶ 293
- “Given all of these publications and their data, from the AZL investigators and



others working in the field, designing AONs capable of inducing exon skipping was highly unpredictable as of the filing date of the AZL applications.” *Id.* ¶ 294;

- “And given the unpredictability inherent in this technology, a person of ordinary skill would need to empirically test each and every AON to determine whether it would be capable of inducing skipping.” *Id.* ¶ 296;
- “As a person of ordinary skill in the art would have understood, small changes in length and composition of the AON can convert a skipping inducing AON into one that will no longer induce skipping.” *Id.* ¶ 297;
- “Because different AON chemistries influence binding affinity, each type of AON will have different binding characteristics, even with identical nucleobase sequences. This prevents meaningful extrapolation from one AON to another.” *Id.* ¶ 407;
- “Even making a small number of these compounds represents a massive investment of time and effort. For example, synthesizing a 30-base morpholino AON takes approximately two weeks... *In vitro* transfection and the subsequent RT-PCR study conservatively takes several days. Thus, testing a single morpholino AON would take on the order of 2.5-3 weeks.” *Id.* ¶ 423;
- “As of March 2003, and to some extent still even today, there is a lack of standardized procedures in place for quantifying dystrophin production, which is a recognized obstacle to the development and evaluation of exon skipping AONs.” *Id.* ¶ 459;
- “The type of cells used in such experiments can have an impact on level of skipping that occurs.” *Id.* ¶ 460;
- “As I explained at length in my first declaration... and as I summarize here, exon skipping was and still is a highly unpredictable technology. Even those who are experts in the field of exon skipping and having a level of skill beyond that of a person of ordinary skill cannot fully explain why some AONs induce skipping while others do not, nor, short of testing, can we determine whether or not a particular AON will be effective in inducing exon skipping, even *in vitro*.” Wood 2nd ‘007 Decl. ¶ 31;
- “It is now known that many factors influence the binding of an AON to its target, including AON length, target accessibility, nucleobase sequence, modifications to the chemical backbone, sequence mismatches, and modifications to the internucleotide linkages. Consequently, there is tremendous variability and unpredictability in the

efficacy of different AONs targeted to different regions of the dystrophin pre-mRNA, and each different AON needs to be empirically tested for its ability to induce exon skipping.” *Id.* ¶ 32;

- “Given all of these publications and their data, from the AZL investigators and others working in the field, designing AONs capable of inducing exon skipping was highly unpredictable. There was, and still is, nothing routine or ordinary about designing an AON or modifying a given AON’s sequence to provoke skipping of any particular exon in an RNA sequence.” *Id.* ¶ 38.
- “In addition to the unpredictability associated with changes in nucleobase sequence ... the secondary and tertiary structure of the pre-mRNA..., the type of cells used in the assessing the ability of the AON to induce exon skipping,the ability to deliver the AON into cells... and the chemical modifications present in the AON can contribute to the ability of an AON to induce exon skipping.” *Id.* ¶ 125;
- “First, the field of exon skipping was exceedingly unpredictable as of 2003, and it remains so today.” *Id.* ¶ 159;
- “exon skipping was a nascent field of study in 2003... It was, and is, difficult to predict which AONs are capable of inducing exon skipping, even *in vitro*.” *Id.* ¶¶ 161-162;
- “It is now known that many factors influence the binding of an AON to its target, including AON length, target accessibility, nucleobase sequence, modifications to the chemical backbone, “mismatches,” and modifications to the internucleotide linkages... Consequently, there is tremendous variability and unpredictability in the efficacy of different AONs targeted to different regions of the dystrophin pre-mRNA, and each different AON must be empirically tested.” ¶ 163;
- “The unpredictability of exon skipping occurs in part because of a “Goldilocks” dilemma. If the AON does not bind tightly enough, exon skipping will not occur. But exon skipping also will not occur if the AON binds *too tightly*, because AON turnover from transcript to transcript is essential... Moreover, because the AON needs to access conformationally complex “target” sequences, changes in length have unpredictable effects.” *Id.* ¶ 164;
- “AONs are complex chemical compounds that can theoretically form Watson-Crick interactions with target pre-mRNA sequences and induce exon skipping. However,

exon skipping is very unpredictable and many complications have prevented successful clinical use of AONs.” *Id.* ¶ 209;

- “It was, and is, difficult to predict which AONs are capable of inducing exon skipping, even *in vitro*.” *Id.* ¶ 215;
- “...small changes in sequence and structure have significant and unpredictable impacts on the ability of an AON to induce exon skipping in the dystrophin pre-mRNA. If an AON does not bind with sufficient affinity, it will not adequately induce exon skipping. If an AON binds too tightly, it also will not induce adequate exon skipping. In the absence of empirical testing, one cannot know whether or not a particular AON would induce skipping, even *in vitro*.” *Id.* ¶ 218;
- Modifications to the nucleobases, chemical backbone, and internucleotide linkages all affect skipping, as does selection of the target tissue... Because different AON chemistries influence binding affinity, each type of AON will have different binding characteristics, even with identical nucleobase sequences. This prevents meaningful extrapolation from one AON to another.” *Id.* ¶ 219
- “Even though Sarepta’s clinical trial data has demonstrated the promise of eteplirsén (a 30-mer PMO), the unpredictability and challenge of successfully delivering exon skipping AONs to treat patients certainly remains true today.” *Id.* ¶ 221⁴;
- “In sum, as of 2003 (and even today), the generation of an AON that induced skipping of exons 51 or 53 was far from routine for a skilled person and would not have been predictable. Because of this lack of predictability, the skilled person would have been required to make and test each AON... to determine whether it was capable of inducing exon skipping.” *Id.* ¶ 222;
- Thus, even *in vitro*, making and testing a single morpholino AON in myotubes (as opposed to rhabdomyosarcoma cells) would have taken on the order of four to five weeks... Because of the unpredictability of exon skipping, practicing the full scope of the claimed invention would require synthesizing and testing each of these

⁴ I wrote this in 2015. As discussed in this report, the promising *in vitro* results for eteplirsén did not translate into efficacy and clinical benefits for DMD patients after eteplirsén received FDA approval in 2016.

compounds. In addition to being prohibitively expensive, this requires a tremendous and undue amount of experimentation.” *Id.* ¶ 237.

15. I note that AZL’s claims were focused on a sequence that was an 18-mer targeting nucleotides +45+62, which is within Dr. Dowdy’s “hot spot.” *See, e.g.*, Dowdy Rebuttal ¶ 355. Thus, Dr. Dowdy is mistaken that the claims in the ‘007 Interference did not target the “hot spot.” Dowdy Suppl. Reb. ¶ 86. My opinions in the ‘007 Interference concerned AONs targeting the “hot spot” and cannot be dismissed as focused on “generalized unpredictability” unrelated to or outside of the exon 53 hot spot. Dowdy Suppl. Reb. ¶¶ 83, 89. In sum, my opinions regarding: 1) the unpredictability of the exon skipping field both before and after June 2005; 2) the effect of small changes to an AON’s composition; 3) the time and effort involved in synthesizing morpholino AONs; and 4) experimental variability that I offered in the ‘007 Interference on behalf of UWA and Sarepta are consistent with those I offer here.

C. The UWA Patents Did Not Disclose a Genus of Exon 53 Skipping AONs

16. In his Supplemental Rebuttal Report, Dr. Dowdy does not substantively dispute that the field of exon skipping was unpredictable. Rather, he asserts that there was predictability within the claimed genus after a POSA read the Wilton Patents. *See, e.g.*, Dowdy Suppl. Reb. ¶ 112 (“the teachings of the Wilton Patents overcame background unpredictability”), ¶ 154 (“the experiments and disclosure of the Wilton Patents addressed the unpredictability in the art with respect to the claimed exon 53 targeting ASOs”); ¶ 175 (“Dr. Wood’s argument is again irrelevant because his generalized concerns are squarely addressed by the Wilton Patents.”). As discussed in my Opening Report (¶¶ 127-131), Rebuttal Report (¶¶ 19-30), and Reply Report (¶¶ 12-29), the teachings of the UWA Patents regarding exon 53 skipping AONs are very limited, and the single figure for exon 53 AONs contains multiple deficiencies undermining its validity and the reliability of the subjective

results reported. In sum, the UWA Patents neither overcame nor addressed the unpredictability of exon skipping, including exon 53 skipping.

1. No POSA would have concluded the UWA Patents empirically demonstrated accessibility of the +23 to +69 region of exon 53

17. Dr. Dowdy contends that the UWA Patents demonstrated “empirically (not through software) that nucleotides 23 to 69 of the exon 53 pre-mRNA are accessible to ASOs and amenable to exon 53 skipping.” Dowdy Suppl. Reb. ¶ 174. This is a variation on his assertion that the UWA Patents disclose a “hot spot” from +23 to +69 within which exon skipping activity was predictable and expected. *Id.* ¶¶ 174-175, 177, 179.

18. I addressed Dr. Dowdy’s “hot spot” argument in other reports. *See, e.g.*, Wood Opening ¶¶ 146-148; Wood Rebuttal ¶¶ 11-74; Wood Reply ¶¶ 6-29; Wood Suppl. ¶¶ 61-76. I also disagree that a POSA would have concluded from the UWA Patents that nucleotides 23 to 69 of exon 53 are accessible to any and all AONs generally, or AONs within the claimed genus in particular. The inventors’ experimental work comprised testing four overlapping 2’-O-Me-PS AONs spanning the +23 to +69 nucleotides of exon 53 for exon skipping. According to Dr. Dowdy, this was sufficient to “establish a hot spot” and that those nucleotides are accessible to AONs, such that a POSA would expect the claimed PMOs to induce exon 53 skipping. Dowdy Suppl. Reb. ¶¶ 62, 174. I disagree. As discussed in my earlier reports, the data on exon 53 skipping in the UWA Patents is weak, inconsistent, and variable. *See, e.g.*, Wood Opening ¶¶ 130-131; Wood Rebuttal ¶¶ 22-27; Wood Reply ¶¶ 19-26. The only figure from the UWA Patents that would allow a POSA to independently evaluate the exon 53 skipping results has multiple technical errors that undermine the validity of the inventors’ methods and their subjective reports of exon skipping. *Id.* These preliminary and low quality results are simply not enough to either establish or allow a POSA to draw conclusions about

accessibility of these nucleotides to AONs or develop expectations about the exon skipping activity of the claimed genus of AONs.

19. Far more work would be required to support a conclusion that a particular region of pre-mRNA is generally accessible to AONs. Popplewell et al., Mol. Ther. 2009; 17(3):554-61 (“Popplewell 2009”), which I discussed previously, is illustrative. The authors of Popplewell 2009 were aware of Dr. Wilton’s work and results with 2’-O-Me-PS AONs. Popplewell 2009, at References (citing multiple publications from the Wilton group). Yet Popplewell undertook to design PMO AONs for exon 53 (and others) essentially from scratch. That design process utilized *in silico* analysis with three different algorithms, an *in vitro* hybridization analysis with synthetic pre-mRNA and an array of all possible hexanucleotide sequences. Based on those results, an array of 25-mer PMOs was designed initially, followed by 30-mer PMOs designed around the target regions of the active 25-mers. Popplewell 2009 at Fig. 1. This is far more experimentation and data than what is reported in the UWA Patents for exon 53. Despite their detailed analysis, the Popplewell 2009 authors did not proclaim that they had overcome the generalized unpredictability in the field or that they had identified a “hot spot.” Rather, they concluded “empirical analysis is still required.” Popplewell 2009 at 559.

20. The Popplewell 2009 authors acknowledged that “[a]ccessibility of the AO to its target site depends directly on the secondary structure of the pre-mRNA, which has a major role in determining AO bioactivity in cells.” Popplewell 2009 at 559. However, Popplewell noted that “[o]verlap of PMO-target sites with open conformations in the folded RNA showed a *weak* association with PMO bioactivity.”⁵ *Id.* (emphasis added). Thus, even accepting that a POSA would

⁵ A later publication by Popplewell et al. (including Dr. Wilton) characterized the “region previously shown to be in open conformation” as +29 to +74, not Dr. Dowdy’s +23 to +69. Popplewell et al., Neuromuscular Disorders 2010; 20(2): 102-110 (“Popplewell 2010”) at 104.

have concluded that the +23 to +69 region of exon 53 was accessible based on a mere four overlapping 2'O-Me-PS AONs reported in the specification (which they would not have done), it does not follow that a POSA would have expected exon skipping to be generally predictable or that AONs targeting that region were likely to induce skipping just because it was accessible. As discussed in my Supplemental Report, exon skipping activity depends not only on target sequence and structure, but also the AON's sequence, length, and chemistry, and a host of extrinsic factors. Wood Suppl. ¶¶ 11-26.

21. Finally, If the UWA inventors had made an important discovery as Dr. Dowdy claims (*see* Dowdy Suppl. Reb. ¶¶ 73, 75, 77, 78), they would have discussed that discovery in their academic publications. However, even after the June 28, 2005 filing date of the UWA Patents, the inventors never even hinted that the +23 to +69 nucleotides of exon 53 was an amenable region for exon skipping that would yield consistent exon removal if targeted. *See* Harding et al., Mol. Ther. 2007; 15(10):157-66 (“Harding 2007”) at Abstract (“no single motif has been implicated in the consistent induction of exon skipping”) and at 163 (“amenable sites to redirect dystrophin splicing were identified at... 39-69 bases within the 212 base long exon 53”); Wilton et al., Mol. Ther. 2007; 15(7): 1288-1296 (“Wilton 2007”) at 1293 (“Retrospective *in silico* studies are under way to determine if there are any trends or features that may assist in future AO design... No single motif emerged as a reliable AO target to bring about consistent exon removal”); Mitrpant et al., J. Gene Med. 2009; 11:46-56 (“Mitrpant 2008”) at 49 (Table 1), 54 (“AO 7 [targeting M53A(+39+69)] *anneals to exactly the same coordinates identified as an optimal target to dislodge human*

However, modelling or predicting RNA folding and open conformations was very difficult and remains difficult today. Popplewell et al. may have shown in an artificial system using a hexanucleotide array that certain regions might be more open than others. It is not surprising that predicted open conformations under such artificial conditions showed a weak association with PMO bioactivity.

dystrophin exon 53...)⁶ (emphasis added) and 55 (“there still appears to be no consistent pattern in AO design for targeted exon skipping”); Mitrpant 2009 at 1422, 1424 (“a *single* AO, H53A(+39+69) was found to induce efficient and specific exon skipping”; these were the “coordinates found to efficiently excise exon 53 from the human dystrophin mRNA”) (emphasis added); WO 2011/057350 (“Wilton PCT ’350”) at 18 (repeating the statements from the UWA Patents’ specification that “[t]he inventors have also discovered that there does not appear to be any standard motif that can be blocked or masked by antisense molecules to redirect splicing”) and 33 (“[a]ttempts . . . to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies.”); Aung-Htut 2019b, at 8 (“We are yet to determine parameters that predict the ideal exon or transcript target for the design of splice modulating AOs...”).⁷ These statements at minimum illustrate the lack of recognition or appreciation by the inventors of the “hot spot,” the claimed genus and any structure-function relationship between the claimed genus and exon 53 skipping. Assuming the inventors had any such recognition in the first place they would also amount to a repudiation of the same.

⁶ The annealing coordinates +39 to +69 are not within the target regions covered by the UWA Patents’ claims. See Dowdy Suppl. Reb. ¶ 16. The Mitrpant 2008 authors noted that “several other AOs overlapping AO 7 also induced this pattern of [exon 53 and 54] removal” but rather than concluding they had shown the existence of a hot spot in mouse exon 53, merely stated their data “impl[ied] that this area may be involved in coordinated processing of both exons during pre-mRNA splicing, at least in mice.” Mitrpant 2008 at 54. This article also uses the term “hotspot” to refer to the region between exons 45 and 55 where deletions are particularly common, consistent with how that term is often used in the DMD field—not how Dr. Dowdy uses it. *Id.* at 47, 52, 55; compare Wood Opening ¶ 51 with Dowdy Suppl. Reb. ¶ 118.

⁷ The only exon 53 AON discussed in Wilton 2007 is H53A(+39+69).

22. Other researchers in the field similarly did not recognize the UWA Patents disclosed a “hot spot” or a genus of exon 53 skipping AONs, or a structure-function relationship. Researchers at Leiden University and Prosensa specifically analyzed the sequences from the UWA Patents specification and results reported in the literature by Dr. Wilton’s group. Aartsma-Rus et al., *Oligonucleotides* 2010; 20(2) (“Aartsma-Rus 2010”) at 2. This analysis attempted to model and predict target regions for effective AONs. *Id.* at Abstract. In essence, the authors were looking for amenable regions for exon skipping, including within exon 53. Yet this retrospective analysis of Dr. Wilton’s data, including data additional to that in the patent specification, did **not** report that Dr. Wilton had discovered or described an exon 53 “hot spot” or specific amenable region. The authors identified the “optimal target” for exon 53 to be “Donor **Exonic**.” *Id.* at Table 7. “Exonic” meaning exon-internal (*i.e.*, every exonic sequence that does not include or overlap with an acceptor or donor splice site) and donor meaning the donor splice site. Notably, the Aartsma-Rus authors did not further specify an amenable region within exon 53, nor acknowledge an exon 53 “hot spot” in any way. Further, the Aartsma-Rus authors interpreted Dr. Wilton’s data as indicating target sequences towards the end of exon 53 at least partially overlapping the donor splice site were also “optimal.” However, Dr. Dowdy’s “hot spot” is closer to the acceptor splice site of exon 53 than the donor.

23. In sum, the real-world evidence reflects that no one working in the field, not even the inventors, came to the same conclusions as Dr. Dowdy—who, as discussed above, was not working in the field—has in this litigation regarding the disclosures of the UWA Patents.

2. **Experimental variability has a significant impact on predictability and burden**

24. Dr. Dowdy criticizes Dr. Hastings and I for conflating unpredictability with experimental variability. Dowdy Suppl. Reb. ¶¶ 126, 151, 177-178. I did not conflate the two. Rather, I described how both intrinsic and extrinsic factors affect exon skipping activity, as well as

the detection of exon skipping activity. Wood Suppl. ¶¶ 22-26. These many factors that influence the outcome of an exon skipping experiment indicate complexity and a correspondingly low degree of predictability. The unpredictability I addressed in my expert reports is not a matter of predicting “the precise amount of exon 53 skipping from one experiment to another.” Dowdy Suppl. Reb. ¶ 177. Rather, it is the fundamental lack of clarity as to how exon skipping actually occurs which precludes the ability to predict results in the absence (or near-absence) of empirical data.

25. Experimental variability is distinct from unpredictability. For example, if a POSA designed an assay to test PMOs for exon 53 skipping that yielded the same “read out” every time, thereby minimizing experimental variability, that would not give them the ability to predict results for a different, untested AON. *See* Wilton 2005 at 225 (“our experience in designing AOs for dystrophin exon skipping that moving an oligonucleotide target site by only a few nucleotides can alter the biological effect by an order of magnitude”). However, where the data generated is variable, *i.e.*, where the signal or output is barely detectable or non-quantitative, or inconsistent across experiments or different assays, that compounds unpredictability. Without reproducible, objective, and quantifiable data, a POSA cannot draw conclusions about whether a given AON does in fact induce exon skipping or predict whether it will induce skipping in the next experiment or a different assay type, much less predict whether a *different* AON will induce skipping.

26. Here, both unpredictability and experimental variability directly inform whether a POSA could reasonably predict, expect, or determine whether any species within the claimed genus would induce exon skipping. As discussed below, and as Dr. Dowdy concedes, there is no guidance in the UWA Patents’ specification as to how to control for extrinsic factors such as delivery methods, and appropriate assays, and reliably test morpholinos for exon 53 skipping. Dowdy Suppl. Reb. ¶¶ 188, 192, 195. Dr. Dowdy agrees that a POSA would have to rely on the general knowledge (which

was scant as of June 2005) and their own experimentation. *Id.* Thus, the UWA Patents leave a POSA no better equipped to identify species within the claimed genus than the UWA inventors were when they first set out to design and test exon 53 skipping AONs.

27. Further, the UWA Patents report no data whatsoever on any species within the claimed genus, only a pseudo-species.⁸ In the absence of data—in what Dr. Dowdy agrees is a generally unpredictable field—no reasonable POSA as of June 2005 would have had any expectations whatsoever regarding the exon skipping activity of the claimed but untested PMOs based on the UWA Patents’ testing of a one pseudo-species. This applies even to a PMO with the same annealing coordinates because AON sequence alone does not determine exon skipping activity. *See Wood Supplemental ¶¶ 12-26.* The inventors also recognized, consistent with my opinion, that the target region is not determinative of exon skipping: “[t]he inability of some splice sites or motifs to respond consistently to AO intervention suggests that other factors, including binding of protein factors, pre-mRNA, or AO secondary structures, may prevent the AOs from annealing and redirecting splicing.” *Wilton 2007 at 1293.*

28. Experimental variability also directly affects the amount of experimentation needed to make, test, and draw reasonable conclusions about exon skipping activity of any individual AON. For example, experimental variability can mean not just different levels/degrees of skipping, but a no skipping result in one assay but some skipping in another. When experimental variability is high, a POSA would not be able conclude whether either a negative or a positive exon skipping result was a

⁸ As in my Supplemental Report, I use the term “pseudo-species” to refer to an AON that meets the length and sequence limitations of the UWA Patents, but is either not a morpholino with thymines instead of uracils or is inactive. Such AONs are not true species of the claimed genus because the claim requires: i) morpholino backbone chemistry; ii) thymines instead of uracils; and iii) exon 53 skipping activity. By way of example, SEQ ID NO: 195, with an annealing site of H53A(+23+47) is a pseudo-species because it is not a morpholino and has uracils, not thymines.

true result if it is not reproducible. *See* Aung-Htut 2019b at 8 (“we suggest that before any AO is classified as ineffective in modulating splicing, various transfection methods should be evaluated”). Repeating experiments in an effort to establish reproducibility increases a POSA’s experimental burden. Thus, experimental variability is highly relevant to the amount of experimentation required to assess the exon skipping activity of AONs encompassed by the UWA Patents’ claims, contrary to what Dr. Dowdy asserts. Dowdy Suppl. Reb. ¶ 151.

29. Dr. Dowdy also does not appear to dispute that “‘candidate’ ASOs” that meet the structural limitations of the claims only “potentially fall within the claim scope” and must be tested to “confirm that exon 53 skipping was actually induced” such that these candidates “*actually* fall within the claim scope.” Dowdy Suppl. Reb. ¶¶ 118; 149 (“The claims simply require a POSA to confirm whether a given PMO ‘induces exon 53 skipping.’”). In view of the unpredictability of the field coupled with significant experimental variability and lack of guidance in the specification as to how to test PMOs for exon skipping at all, the experimental burden on a POSA to test ten or more PMOs for exon skipping would have been very heavy and would have taken far longer than the “matter of weeks” according to Dr. Dowdy. *Id.* ¶ 166.

3. The UWA Patents did not advance or accelerate research in the field

30. In my opinion, the approach taken by those working in the DMD field after June 2005—including Dr. Wilton himself—reflects a lack of recognition or appreciation that the UWA Patents disclosed a relationship between the parameters of the genus as set forth in claim 1 of the ‘851 patent and exon skipping function. Wood Suppl. ¶ 76. As I stated, if the inventors had in fact identified and sufficiently disclosed such a structure-function relationship, the rational approach for them and others in the field would have been to focus on the claimed genus in order to expeditiously develop optimal therapeutic candidates. Wood Suppl. ¶ 66. Dr. Dowdy acknowledges that

“optimizing skipping efficiency” to identify “optimal clinical candidates is a goal that POSAs had.” Dowdy Suppl. Reb. at ¶ 223.

31. Dr. Dowdy criticizes my premise as speculation. Dowdy Suppl. Reb. ¶ 223. It is not speculation, it is logic. The only speculative aspect is that the UWA Patents provided sufficient disclosure to direct a POSA towards the claimed genus of exon 53 skipping AONs. Regardless, despite his criticism, Dr. Dowdy apparently *agrees* that a POSA *would* take the rational approach I outlined. For example, Dr. Dowdy states (emphasis added):

- “Of course, in reality, a POSA would not have synthesized every possible ASO within the scope of the claims, but *would have engaged in a rational course of experimentation to test* a smaller number of *ASOs having the claimed structural features* to streamline the process” (¶ 54)
- “even if some confirmatory experimentation would be needed, a POSA *would have prioritized candidate AOs* and then rationally focused on groups of ASOs”⁹ (¶ 136)
- “A POSA *would have evaluated* the target regions encompassed by the Wilton Patents using a small number of *candidate PMOs*.” (¶ 169)

32. As discussed in my Supplemental Report, publications on exon 53 skipping show that those working in the DMD field after the disclosures of the UWA Patents did not in fact start with “a smaller number of ASOs having the claimed structural features” or “prioritize candidate ASOs” or focus on “candidate PMOs” as would have been logical had the UWA Patents disclosed such candidates. See Wood Suppl. ¶¶ 61-76. Not even Dr. Wilton took that approach in designing additional exon 53 AONs after the initial set published in the UWA Patents as would have been logical had he recognized or appreciated the claimed genus in June 2005. Wood Suppl. ¶ 65. Dr. Dowdy does not dispute that only a small fraction (21 out of 167) of the exon 53 AONs designed and tested after June 2005 were species of the claimed genus.¹⁰ Wood Suppl. ¶¶ 61-76.

⁹ Dr. Dowdy uses the term “‘candidate’ ASOs” to refer to those that “potentially fall within the claim scope of the Wilton Patents,” e.g., potential species. Dowdy Suppl. Reb. ¶ 18.

¹⁰ Dr. Dowdy does not dispute my classification of those AONs as species, pseudo-species, or not species.

33. Given that Dr. Dowdy agrees with my premise—that a POSA would have focused on species within the claimed genus if the UWA Patents had indeed disclosed a set of structural features that confer exon skipping activity—and does not dispute my analysis of AONs as species vs. not-species, he should also agree with my conclusion that the real-world evidence shows the **lack** of contemporaneous recognition and appreciation of the claimed genus by Dr. Wilton and others working in the field. He does not. Instead, Dr. Dowdy dismisses as “irrelevant” whether researchers “test[ed] additional ASOs outside the exon 53 hot spot” or “test[ed] additional ASOs within the hot spot to optimize skipping efficiency.” Dowdy Suppl. Reb. ¶¶ 222-223.

34. At the same time, Dr. Dowdy proclaims the “importance of the claimed inventions” and “the importance of the exon 53 hot spot” disclosed in the UWA Patents specification. *See, e.g.*, Dowdy Suppl. Reb. ¶¶ 73, 75, 77. Contradicting their alleged importance, the real-world evidence reflects that the UWA Patents had **no apparent impact** on how the field (including Dr. Wilton) approached designing AONs for exon 53. Wood Suppl. ¶¶ 61-76. Contrary to Dr. Dowdy’s claim (Dowdy Suppl. ¶ 135), the alleged disclosure of the structure-function relationship between the claimed genus and exon 53 skipping activity did not eliminate or significantly reduce the amount of experimentation that later researchers undertook to identify effective exon 53 skipping AONs. Wood Suppl. ¶¶ 61-76

35. Indeed, the only contribution of the UWA Patents to the field that Dr. Dowdy identifies is that other researchers used the H53A(+39+69) sequence as a positive control.¹¹ Dowdy Suppl.

¹¹ I disagree with Dr. Dowdy that the other researchers used H53A(+39+69) as a “positive control.” Rather, the publications indicate the sequence was used as a comparator. *See* Popplewell 2010 at 107 (“sequences were compared to”); Watanabe PCT ‘318 at [0314] (The PMOs of the invention “caused exon 53 skipping with a markedly high efficiency... as compared to the oligomers... in accordance with the prior art.”); Sazani PCT ‘586 at 76:7-11 (“Other sequences were compared... including H53A(+39+69)”).

Reb. ¶ 224. However, this sequence is *not* a species of the claimed genus because it does not meet the structural requirements.¹² A recognition that one AON that is not a species might serve as a positive control is not recognition or acknowledgement of a “hot spot” of +23 to +69 of exon 53, or, more importantly, of a relationship between the structural requirements of the UWA Patents’ claims and exon 53 skipping function. It is unclear if other researchers even relied on the UWA Patents’ report on this AON, as opposed to later publications from the Wilton lab that identified this target region/AON as the “amenable site” within exon 53 to redirect splicing (Harding 2007 at 163), the “AO[] that proved to be most effective at inducing exon [53] removal” (Wilton 2007 at 1293), “an optimal target” for exon 53 (Mitrpant 2008 at 54) and the “single AO” that induced efficient and effective exon skipping from the “initial draft of AOs targeting human dystrophin exons 2-78” tested (Mitrpant 2009 at 1422, 1418).

36. The only reasonable conclusion from this real-world evidence is that the UWA Patents neither disclosed an exon 53 hot spot nor described a set of structural features that conferred exon 53 skipping activity—because the inventors did not recognize or appreciate such an invention. Dowdy Suppl. Reb. ¶ 230. As reflected by how researchers working in the field approached designing exon 53 AONs after June 2005, the UWA Patents at best describe a first small step in a long process towards developing therapeutic exon 53 skipping AONs.

4. I have not acknowledged Dr. Dowdy’s exon 53 “hot spot”

37. I previously addressed Dr. Dowdy’s contentions that the UWA Patents identified a “hot spot” within exon 53. *See, e.g.,* Wood Rebuttal ¶¶ 11-69; Wood Reply ¶¶ 12-29. Dr. Dowdy now claims that I recognized and acknowledged the “hot spot” outside of this litigation. Dowdy

¹² Dr. Dowdy also holds the opinion that AONs outside the scope of the asserted claims are “irrelevant.” Dowdy Suppl. Reb. ¶¶ 129-130.

Suppl Reb. ¶ 225. I disagree with Dr. Dowdy's interpretation of the patent applications and my correspondence with Dr. Wilton.

38. The PCT applications Dr. Dowdy points to, WO 2022/171972 ("Wood PCT '972") and WO 2022/172019 ("Wood PCT '019"), are directed to cell-penetrating peptide conjugates and methods of their use. The inventions described in these PCT applications address the limitation of AONs as DMD therapy, namely the relatively inefficient targeting of skeletal muscle, as well as the inadequate targeting of single stranded oligonucleotides to other affected tissues such as the heart. *See* Wood PCT '972, at 1. For example, the level of dystrophin restoration achieved by Sarepta's eteplirsen product, which is approved for boys with mutations amenable to exon 51 skipping, is disappointing, at a reported approximately 1% of normal dystrophin levels. Based on Becker Muscular Dystrophy and data generated from *mdx* mice, at least ~15% of wild-type dystrophin levels is needed to protect muscle against exercise induced damage. *Id.* Thus, the peptide conjugates described in the PCT applications are intended to enhance the ability of AONs, including FDA-approved AON sequences, to penetrate cells, and thereby enhance their ability to induce exon skipping, and improve their therapeutic efficacy. Eteplirsen in particular suffers from an apparent lack of efficacy. Thus, there is a need to improve delivery of AONs, which the peptide conjugates are intended to meet.

39. Table 3 of the PCT applications provides "non-limiting examples" of sequences that are "capable of inducing exon 53 skipping." Wood PCT '972 at 27. I have reviewed the sequences of Table 3, and the sequences listed are from the 2011 PCT application filed by Watanabe et al. in, PCT/JP2011/070318, published as US 2013/0211062, plus the H53A(+39+69) sequence published by Dr. Wilton. Wood PCT '972 at 27-28, Table 3; Wood PCT '019 at 30-31, Table 3. Several of the sequences from Table 3 are not species because they are either too short or too long. As I discussed

in my Supplemental Report, Watanabe et al. screened the entirety of exon 53 in order to identify the most active AONs. Wood. Suppl. ¶¶ 72-74. Citing prior art patents and listing previously published sequences represents, as Dr. Dowdy put it, “building upon prior studies, which is common in the DMD field “as in any field of study.” Dowdy Suppl. Reb. ¶ 224. Regardless, listing one sequence published by Dr. Wilton’s group (that is not a species of the claimed genus) along with other AONs published by others is not recognition or acknowledgement the UWA Patents disclosed a “hot spot,” a genus of exon 53 skipping AONs, or a structure-function correlation between the claimed genus and skipping activity.

40. Finally, my 2011 email correspondence with Dr. Wilton is not an acknowledgement of an exon 53 hot spot. *Cf.* Dowdy Suppl. Reb. ¶ 226. That series of emails began with Dr. Dickson inviting Dr. Wilton to suggest one or two sequences for a comparative study comprising an ““even more detailed base-by-base stepped screen of the target region to complement the published optimization.””¹³ WILTON0017612 at -616. Dr. Wilton identified his best two exon 53 AONs as “having the annealing coordinates +33+63 and +33+65.” *Id.* at -615. The former could be a species of the claimed genus if made as a PMO and tested for exon 53 skipping activity; the latter could not be a species because it is too long. *See* Wood Suppl. Reb. ¶ 65. Dr. Wilton states that the *non-species* “seems to still be more active at lower concentrations but there is not much in it.” *Id.* (emphasis added). In my response, I thanked Dr. Wilton for sharing his sequences, and wrote “it would be good to evaluate these sequences as PMOs soon.”¹⁴ *Id.* at -614. Consistent with my opinions in this

¹³ I infer from the date and context that this statement refers to Popplewell 2010. Thus, the Popplewell authors intended to take up their own suggestion that “a stepped base-by-base screening of AOs across the entirety of exon 53” might reveal a more effective AON. Popplewell 2010 at 108.

¹⁴ Dr. Dickson indicated that the PMOs for the study would be obtained from Gene Tools. WILTON0017612 at -616.

litigation, I did not accept that results with a 2'O-Me-PS AON would necessarily translate to the corresponding PMO even years after June 2005 with the benefit of Popplewell et al.'s publications.

D. A POSA Could Not Reasonably Have Tested the Full Scope of the Claimed Genus

41. The resources required to make and test every AON within the claimed genus would have been a prohibitive barrier to actually doing so. Dr. Dowdy's assertion that testing 168 PMOs was "equivalent to a short project that any graduate student in my laboratory could have accomplished" (Dowdy Suppl. Reb. ¶ 166) is not supported. It requires accepting that a POSA—a person of *ordinary* skill—had knowledge and skills *exceeding* that of the UWA Patents' inventors and other inventors working in the field as well as unlimited resources in terms of reagents, laboratory equipment, personnel, and time.

42. Dr. Dowdy's assertion that the UWA Patents provide "extensive guidance" on how to obtain, test, and "confirm" exon 53 skipping (Dowdy Suppl. Reb. ¶ 168) is also not supported. As I noted in my Supplemental Report, there is *no* guidance in the UWA Patents' specification regarding how to test morpholino AONs for exon skipping. Wood Suppl. ¶ 38. In rebuttal, Dr. Dowdy has not pointed to any passage *in the UWA Patents' specification* that discusses or provides guidance regarding morpholinos, other than the header in Table 1A that states "U bases may be shown as 'T'" for "morpholinos." See Dowdy Suppl. Reb. ¶¶ 105, 111, 156. Dr. Dowdy also agrees with me that the transfection conditions disclosed in the UWA Patents' specification "did not concern PMO delivery." *Id.* ¶ 190. Thus, Dr. Dowdy relies on what he claims a POSA would have known and could have done rather than the UWA Patents' specification, because that information is entirely missing from the specification itself. *Id.* ¶ 189. However, Dr. Dowdy's opinions regarding what a POSA would have known and could have done are contradicted by what researchers working in the field *actually did*.

1. Synthesizing PMOs Was No Ordinary Task

43. The definition of a POSA does not include knowledge or experience in organic chemistry or in synthesizing antisense oligonucleotides (for exon skipping applications or otherwise) that would be required to synthesize morpholinos themselves as opposed to purchasing from a vendor. Dowdy Suppl. Reb. ¶ 138. Consistent with the POSA definition, the literature reflects that actual researchers working in the field did not synthesize PMOs themselves, but rather purchased them from Gene Tools. This includes the only two laboratories that had published on two PMOs for exon skipping at the time: Dr. Wilton's group at UWA, and the group working at AZL (also known as Leiden University) which included Drs. Aartsma-Rus, van Deutekom, and van Ommen. Gebski et al., Hum Mol Gen 2003; 12(15): 1801-11 ("Gebski 2003") at 1809 (the one morpholino AO was "purchased from Gene Tools, USA"); Aartsma-Rus 2004 at 1396. Thus, even persons of *extraordinary* skill in the field of exon skipping ASOs, such as inventors Drs. Wilton and Aartsma-Rus, did not synthesize their own PMOs. Nor have they done so to this day as far as I am aware.

44. The other references Dr. Dowdy cites as evidence of successful delivery of PMOs or testing of multiple PMOs similarly reflect that scientists working with PMOs before and during the 2005 time frame were either affiliated with Gene Tools or AVI BioPharma (e.g., James Summerton, Pat Iversen), or obtained their PMOs from one of those companies. Dowdy Suppl. Reb. ¶¶ 188, 197, 209 (citing Schmajuk 1999 ("morpholino oligonucleotides were a gift from Antivirals and Gene Tools companies") at 21784; Nasevicius & Ekker 2000 at 219 ("Morpholinos were obtained from Gene Tools, LLC"); Sazani 2001 at 3966 (listing James Summerton as a co-author and stating that the two morpholinos tested were "synthesized and described elsewhere"); Sazani 2002 at 1229, 1232 (the two morpholino oligomers tested were "obtained from Gene-Tools"); Deere 2005 at 249 (all authors were from AVI Biopharma and "PMOs were synthesized and purified at AVI Biopharma")).

45. The real-world evidence of how scientists testing PMOs generally, and scientists working in the field of exon skipping specifically, obtained their PMOs as of June 2005 reflects that Gene Tools was the sole commercial source of PMOs and that a POSA would not have simply synthesized them in-house one at a time, much less “in parallel and in an automated fashion.” Dowdy Suppl. Reb. ¶ 162. For example, Moulton, Curr. Prot. Nucleic Acid Chem. 2006 (“Moulton 2006”), an article on how to design morpholino experiments, instructs scientists to “order the synthesis of the selected Morpholinos” and identifies Gene Tools as the “[c]ommercial source for morpholinos.” Moulton 2006 at 4.30.1 and 4.30.24.

46. With respect to hypothetical POSAs working in industry who might have had more extensive resources (Dowdy Suppl. Reb. ¶ 145), Dr. Summerton’s U.S. Patent No. 5,185,444, which claims morpholino polymers, and other Gene Tools or AVI Biopharma patents, could have discouraged companies from synthesizing their own morpholinos. For example, Prosensa, a pharmaceutical company established in 2002 that was working on developing exon skipping AONs for DMD in collaboration with researchers at AZL focused on 2’O-Me-PS AONs.¹⁵ And Ionis Pharmaceuticals (then known as Isis Pharmaceuticals) focused on 2’O-MOE AONs. Thus, Dr. Dowdy’s assertion that the “POSA is not limited to researchers in academic laboratories” to support his argument that POSAs at pharmaceutical companies could have or would have synthesized morpholinos themselves fails to account for the real-world evidence and for relevant IP considerations.

¹⁵ Contrary to Dr. Dowdy’s claim that “a POSA as of June 2005 would have focused on [PMO] oligomers” (Dowdy Suppl. Reb. ¶ 28, n.4), Prosensa and the Dutch researchers continued to focus their efforts on the 2’-O-Me-PS AONs for years after June 2005, with Prosensa/Biomarin applying for approval of drisapersen, a 2’-O-Me-PS. The FDA rejected that application in 2016.

47. In sum, Dr. Dowdy's assertion that a POSA would have made or been able to make their own PMOs is contradicted by the real-world evidence that the scientists working in the field as of 2005 *did not do so*. Dowdy Suppl. Reb. ¶¶ 143-144. Again, the barrier was not just the expense of reagents and equipment, but also the expertise and experience required. Even if a POSA worked in a laboratory with the financial, physical, and human resources required to synthesize a PMO, the time it would have taken to synthesize PMOs against each of the 168 unique target regions of the claimed genus was prohibitive and undue. Contrary to Dr. Dowdy's unreasonable belief, it would have been a tremendous, if not insurmountable, burden on a POSA to make even a fraction of the PMOs encompassed by the UWA Patent claims, which includes PMOs with nucleotide substitutions and end cap modifications.

48. Dr. Dowdy asserts without any supporting evidence that this synthesis "would take at most a few weeks." Dowdy Suppl. Reb. at ¶ 161. He criticizes my and Dr. Hastings' reliance on a 2021 publication by Li et al. to show that difficulties involved in synthesizing PMOs continued well beyond 2005. *Id.* at ¶¶ 162, 182. Dr. Dowdy also accuses me of misquoting Li et al., Nature Communications 2021; 12:4396 ("Li 2021"). Dowdy Suppl. Reb. ¶ 182, n. 14. I disagree. It is Dr. Dowdy who appears to have misunderstood or misinterpreted the fundamental improvements over existing PMO synthesis methods described by Li 2021.¹⁶ The publication illustrates that, as of 2021, it took 180 minutes *per nucleotide* to synthesize a PMO. Li 2021 at 7 (we "ultimately succeeded in decreasing coupling time from 3 h per nucleotide to 8 min."). This is consistent with the authors' statement that it took "on the order of weeks" to synthesize *one* PMO sequence of ~20 residues as

¹⁶ Two of the authors on Li 2021 are Sarepta employees, and the paper reports that MIT and Sarepta filed an international patent application covering part of the work reported. As Li notes, "[d]evelopment of PMO drugs requires testing of many sequences [per exon] and laborious production of sequential revisions." Li 2021 at Fig. 1.

opposed to their achievement (in 2021) of synthesizing “three [PMO] candidates for a new DMD treatment in a single day.” Li 2021 at 2, 7.

49. Li 2021’s description of the state of the art in 2021 refutes rather than “reaffirms” Dr. Dowdy’s opinion that “a POSA as of June 2005 would have been able to make PMOs within a few weeks” and specifically the hundreds of “PMOs corresponding to every target region covered by the claims” a large majority of which are longer than 20 bases in length. Dowdy Suppl. Reb. ¶¶ 162, 163. Prior to Li’s improvements, it would have taken at least three *years* to synthesize 168 PMOs of ~20 residues sequentially.

50. Dr. Dowdy’s assertion that a POSA “could make multiple PMOs simultaneously using multiple [automated] synthesizers” is similarly baseless. Dowdy Suppl. Reb. ¶ 163. PMO synthesis is not easily adapted to automated synthesis; apparently the first protocol adapted to synthesize “regular PMOs [with] a commercial DNA synthesizer” was published in **2022**. Kundu et al., J. Org. Chem. 2022; 87:9466-9478 at Abstract, 9467. Even as of 2022, “automated synthesis was not a norm” and PMOs were “only available from the original inventors,” *i.e.*, Gene Tools. *Id.* at 9467, 9473.

51. In June 2005, synthesizing PMOs was difficult and time-consuming. For example, even researchers at AVI Biopharma, which had the ability to synthesize PMOs in-house, tested only tens of PMOs in a study contemporaneous to the UWA Patents. Dowdy Suppl. Reb. ¶ 209 (citing Deere 2005 as testing 56 PMOs).¹⁷ That is an order of magnitude less than the number of PMOs required to test the unique target regions encompassed by the UWA Patents’ claims, and several orders of magnitude less than the total number of species with the nucleotide substitutions and end

¹⁷ Deere 2005 tested the ability of PMOs to inhibit gene expression using a luciferase expression assay in *E. coli* and HeLa cells, and a cell-free system. Deere et al., Antimicrobial Agents and Chemotherapy Jan. 2005; 49(1): 249-255. These PMOs were 7 to 20 bases in length. *Id.* at 251.

modifications that Dr. Dowdy admits are also encompassed by the genus. Dowdy Suppl. Reb. ¶¶ 59-60. Thus, it seems highly unlikely that AVI BioPharma would have been willing or able to supply a research partner with hundreds of different PMOs for testing.

52. In sum, Dr. Dowdy's unsupported assertions that a POSA could make their own PMOs and further could make the large number of "PMOs corresponding to every target region covered by the claims" in "at most a few weeks" (Dowdy Suppl. Reb. ¶¶ 161, 163, 181-182) are unrealistic, unfounded, and contradicted by the real-world evidence of what the handful of scientists testing PMOs for exon skipping in June 2005 in fact did.

2. A POSA could not have purchased PMOs encompassed by the claims

53. Dr. Dowdy asserts that a POSA could purchase the PMOs encompassed by the claims. Dowdy Suppl. Reb. ¶¶ 142, 163. In terms of length, I note that in a later part of the 2011 email correspondence cited by Dr. Dowdy, Dr. Dickson informed Dr. Wilton that the "two AOs you indicate are a 31-mer and a 33-mer, respectively, and correspond to one of the most active 30-mer PMOs (H53A30/2, +33+62) defined in our NMD paper... but simply extended by [1 nucleotide] on the 3' side. *Generally we work only with 30-mer PMOs which is the maximum synthesis size offered by GeneTools.*"¹⁸ WILTON0018478 at -480. Dr. Wilton responded that he would see if he could get the longer PMOs from AVI. WILTON0018492 at -495. Dr. Dowdy states that 31-mer PMOs had been made and tested before June 2005 without citing a publication. Dowdy Suppl. Reb. ¶ 181. If the source of the 31-mer PMOs was AVI Biopharma, a small number of limited, unpublished examples does not establish that POSAs generally could have obtained 31-mer PMOs in June 2005. It was well-known in the field that there were technical challenges in terms of yield and purity, as well as increased costs, to synthesizing PMOs longer than 25-mers.

¹⁸ "Our NMD paper" refers to Popplewell 2010.

54. In any case, this correspondence confirms that Gene Tools “set the limit” at 30 bases and did not sell PMOs 31 bases in length. *See* Wood Suppl. Reb. ¶ 29. Therefore, a POSA would not have been able to purchase PMOs to test nearly 10% of the unique target regions encompassed by the claims of the UWA Patents, much less the myriad of 31-mer variants with nucleotide substitutions or end modifications that are also encompassed by the claims. *See* Dowdy Suppl. Reb. ¶¶ 16, 142.

55. The claims of the UWA Patents do not exclude end modifications. Dr. Dowdy agrees that both 5’ and 3’ end modifications were known in the art as of June 2005. Dowdy Suppl. Reb. ¶ 182. Dr. Dowdy does not dispute that, as of 2006, Gene Tools only offered for sale a limited set of 3’ end modifications. *Id.* ¶ 182, n. 13. Attaching 3’ modifications to an AON must be done batch-by-batch – again, resource-intensive. Not “another few weeks at most” as Dowdy asserts. *Id.* ¶¶ 161, 183.

56. Notably with respect to the 5’ end modifications, Gene Tools stated that “due to synthesis constraints of Morpholino oligos we **cannot** modify the 5’ end of a Morpholino oligo.”¹⁹ Thus, Dr. Dowdy’s assertion that “attaching a modification to the 5’ or 3’ end of a PMO would have been within the skill of a POSA” (Dowdy Suppl Reb. ¶ 182) is contradicted by Gene Tools, which was founded by the co-inventor of PMOs and the sole commercial source of PMOs in June 2005. Accordingly, a POSA would have had no means of obtaining and testing PMOs with **any** of the 5’ end modifications that were known in the art as of June 2005.

57. Dr. Dowdy dismisses end modifications as “peripheral for inducing exon skipping.” Dowdy Suppl. Reb. ¶¶ 44-45. This is simply incorrect. End modifications would be expected to have

¹⁹ <https://web.archive.org/web/20051222184949/http://www.gene-tools.com/node/23> (emphasis added)

some effect whether a given AON would induce exon 53 skipping. Dr. Dowdy concedes that these modifications impact solubility, delivery, cell penetration and uptake. *Id.* ¶¶ 44-45. These properties, particularly delivery, cell penetration, and uptake are essential for successful exon skipping. *See, e.g.,* Aung-Htut 2019b at 8 (“AO delivery is a crucial aspect for splice switching efficacy”); *see also* Wood Opening ¶¶ 99-105. Exon skipping is not an inherent property of any AON. An AON that is not taken up by the cell or distributed to the nucleus cannot induce exon skipping regardless of whether its sequence is capable of Watson-Crick base pairing with the target pre-mRNA.

58. As Gebski 2003 states, “[e]xon skipping is ***dependent*** upon the ability of the AO to reach the nucleus and then interfere with spliceosome assembly.” Gebski 2003 at 1802 (emphasis added). The Gebski co-authors (which included Drs. Wilton and Fletcher) reported that the unleashed morpholino induced no skipping, thereby demonstrating the leash modification was central, not peripheral, to exon skipping. *Id.* at Figs. 2 and 3. McClorey et al. reported similar negative results for uncomplexed PMOs (obtained from AVI BioPharma), and that passive diffusion did not produce increasing levels of exon skipping over time as they had initially hypothesized. McClorey et al., *Gene Ther* 2006; 13:1373-1381 (“McClorey 2006”) at 1379, 1380.

59. With respect to nucleotide modifications, there is no indication that Gene Tools would have been willing to synthesize and sell morpholinos with modified bases as disclosed in Summerton US ‘444. As mentioned above, any change to that chemical makeup, whether changing the sequence, the backbone chemistry, or introducing a nucleotide modification or an end modification, could affect its exon skipping activity. Given the limited knowledge concerning unmodified, natural base PMOs in the exon skipping field in June 2005, a POSA would have no way to predict how such chemical and structural variations would affect exon skipping. Even holding all variables constant and

synthesizing only PMOs with natural bases and 100% complementary to the 168 unique target regions of the '851 patent would have been an impossible undertaking in June 2005.

60. Dr. Dowdy contends that “a POSA reading the Wilton Patents would have understood the term ‘morpholino antisense oligonucleotide’ to refer to a PMO, not an oligomer with different intersubunit linkages.” Dowdy Suppl. Reb. ¶ 24. Although the term “morpholino” is often used interchangeably with PMO, there are examples in the contemporaneous literature where it is not. *See, e.g.*, U.S. Patent No. 6,784,291 to Iversen and Hudziak and assigned to AVI BioPharma Inc. at col. 3:56-4:3 (defining “morpholino oligomer” to include multiple intersubunit linkages). The references cited by Dr. Dowdy comprise the Summerton & Weller 1997 publication and references that cite to that publication. However, as stated in my Supplemental Report, Summerton & Weller 1997 expressly describes other intersubunit linkages. Wood Suppl. ¶ 83.

3. Methods for testing PMOs were not well-established

61. As I discussed in my Supplemental Report, delivering morpholino AONs to cells was a known challenge, and the information available to a POSA as of June 2005 on techniques to overcome that challenge was limited. Wood Suppl. ¶¶ 34-43. General approaches for other cell types were known, but specific methods for achieving consistent delivery of PMOs for exon skipping assays had not been established as of June 2005. They certainly were not “well known in the art” at that time as Dr. Dowdy claims. Dowdy Suppl. Reb. ¶ 189.

62. In the only two published studies, researchers explored the use of leashes. Gebiski 2003; Aartsma-Rus 2004. Those leashes were designed for AONs targeting mouse exon 23 and human exon 46, not human exon 53. Thus, a POSA could not simply use the leash disclosed by Gebiski to test exon 53 AONs. Dr. Dowdy contends that a POSA would have been able to “design

adequate leashes” for exon 53 based on the teachings of Gebski 2003 and Aartsma-Rus 2004.²⁰ Dowdy Suppl. Reb. ¶ 148. I note that the Aartsma-Rus 2004 authors—whose skills as inventors in the field exceeded that of a POSA—were aware of Gebski 2003, yet their resulting leash was not optimal. *See* Dowdy Suppl. Reb. ¶ 96. The “technical errors” of Aartsma-Rus 2004 illustrate the nascent state of the art and the issues that persons of extraordinary skill encountered testing PMOs. Dowdy ¶ 153 (describing experimental conditions used as “suboptimal”). Even assuming a POSA would have known how to design “adequate leashes” for exon 53 PMOs, those new leashes would need to be tested for efficacy, which the inventors called a “complex preparation” (McCloreay 2006 at 1378), thereby adding to a POSA’s experimental burden.

63. Alternatively, Dr. Dowdy suggests that a POSA would have used Endo-Porter, a reagent sold by Gene Tools, or cell-scraping, or other techniques used outside of the exon skipping field. Dowdy Suppl. Reb. ¶¶ 148, 186. This assertion ignores that no one had reported using Endo-Porter or cell scraping for exon skipping assays as of June 2005. Cell scraping works only *in vitro*, and there were concerns that these agents might not perform well or would be toxic *in vivo*.²¹ *See, e.g.*, Gebski 2003 at 1809 (“the requirement for delivery reagents contribute their own disadvantages, such as potential toxicity and/or modified tissue distribution and potential serum and cell interactions.”); Wilton & Fletcher, *Acta Myologica* 2005; XXIV:222-229 (“Wilton 2005”) at 226; McCloreay 2006 at 1378 (“in terms of a clinical application, it would be preferable to use a chemistry

²⁰ Elsewhere, Dr. Dowdy dismissed data generated for other exons as “immaterial” and “irrelevant” when Dr. Hastings cited it to illustrate unpredictability. Dowdy Suppl. Reb. ¶¶ 83, 119.

²¹ As of June 2005, the safety and efficacy of Endo-Porter was unknown; the article reporting that Endo-Porter was safe and effective *in vitro* was published in November 2005. Summerton, *Ann. N.Y. Acad. Sci.* 2005 (Nov); 1068:62-75 (“Summerton 2005”). Even after June 2005, the researchers who selected and developed the first PMO exon skipping drug did not select Endo-Porter as a transfection reagent (with leashed PMOs). Archevala-Gomez 2007 at 805 and Fig. 4.

that did not require a transfection agent, as a limitation of using cationic liposomes as a carrier is associated toxicity and aggregation with serum proteins *in vivo*.”); *see also* Wood Opening ¶ 73.

64. Again, the ultimate goal of a POSA was to develop a therapeutic AON for delivery to DMD patients. None of these above delivery agents or methods—leashes, scraping, Endo-Porter, cationic liposomes—were applicable to delivering exon skipping PMOs *in vivo*. Their use in preclinical work would add a very significant complication because of this lack of clinical translatability to use in DMD patients.

65. In sum, there was limited guidance and knowledge in 2005 that a POSA could rely on to design reliable assays to test PMOs for exon skipping, including exon 53 skipping. Ensuring efficient and consistent uptake was a “major limitation[] in evaluating PMOs in cultured cells at the time. McClorey 2006 at 1374. Similarly, my co-authors and I noted that despite numerous *in vitro* exon skipping studies (including several published after 2005) “little agreement exists on the optimal parameters for *in vitro* AO screening”—including of PMOs. Wang et al., J Gene Med 2010; 12(4):354-64, at 362. The papers Dr. Dowdy cites as providing “detailed protocols for conducting exon skipping assays” were not for PMOs. Accordingly, developing reliable, predictable methods and assays to test the exon 53 skipping activity of PMOs would have increased the burden on a POSA to determine whether AONs meeting the structural limitations had exon skipping activity.

E. A POSA Would Not Have Extrapolated Results From the One Pseudo-Species in the UWA Patents to the Entire Claimed Genus

66. An AON’s chemical makeup “affects pairing stability, metabolic stability, protein binding and toxicities, altered coagulation, immune cell and/or complement activation, off-target effects, pharmacokinetics and pharmacodynamics.” Egli & Manoharan, Nucleic Acids Res 2023; 61(6):2529-73 (“Egli 2023”) at 2537. Any change to that chemical makeup, whether changing the sequence, the backbone chemistry, or introducing a nucleotide modification or an end modification,

could affect exon skipping activity. In particular, changes to backbone chemistry affect stability (both in terms of resistance to nucleases and thermostability), how the AON binds to RNA and interacts with protein, how the AON enters cells, exits endosomes, is trafficked to the nucleus, etc.

67. Dr. Dowdy asserts that “it was known that the exon skipping ability exhibited by a 2’OMePS ASO is generally translatable to a corresponding PMO targeting the same region.” Dowdy Suppl. Reb. ¶¶ 93, 104, 153, 156-157, 203-204. He supports this assertion with only three references that were published prior to the June 28, 2005 priority date, Gebski 2003 and Aartsma-Rus 2004, and Morita 2003 (which did not address exon skipping). *Id.* However, these references do not support Dr. Dowdy’s assertion—either generally, or specifically with respect to exon 53.

68. As I mentioned in my Supplemental Report, the PMO tested in Gebski 2003 was directed to mouse exon 23, differed in sequence and length from the preceding 2’-O-Me-PS AON, and was annealed to a leash. Wood Suppl. ¶¶ 39-40. Thus, Gebski 2003 cannot support Dr. Dowdy’s assertion because it is not a direct comparison of the relative skipping activities of a 2’-O-Me-PS AON and the corresponding PMO.

69. The authors of Aartsma-Rus 2004 concluded that the 2’-O-Me PS AON backbone was “currently the most favorable compounds, at least for targeted DMD exon 46 skipping.” Aartsma-Rus 2004 at Abstract. Nevertheless, Dr. Dowdy contends that Aartsma-Rus 2004 “would buttress the expected transferability between 2’OMePS and PMO ASOs” based on the statement “[f]urther optimization of the morpholino itself and the leash required for EPEI [ethoxylated polyethylenimine] transfection may thus increase the levels of exon... skipping.” Dowdy Suppl. Reb. ¶ 153 (quoting Aartsma-Rus 2004 at 1396). As the wording makes clear, the authors were merely hypothesizing that

further optimization of the morpholino sequence and leash *may* increase the levels of exon skipping. Further, that hypothesis was specific to *exon 46*... a detail that Dr. Dowdy's quote elides.²²

70. Regardless, this is not an affirmative statement that exon skipping results with a 2'-O-Me-PS AON can be or should be imputed to the corresponding PMO. Nor does it express an expectation that the effect of the *same* PMO "would be greater with appropriate transfection conditions" contrary to Dr. Dowdy's assertion. Dowdy Supp. Rep. ¶ 153. The authors could not distinguish between the potential causes of the low levels of exon skipping observed. Aartsma-Rus 2004 at 1396 ("the low levels of exon skipping *may* be the result of poor nuclear uptake rather than the low efficiency of the morpholino" and suggesting optimizing sequence and the leash "*may* increase levels of exon 46 skipping") (emphasis added).

71. That Aartsma-Rus et al. undertook such a comparative study reflects that the relative skipping efficiencies of the different AON analogs was an open question at the time. Further, the "technical limitations" Dr. Dowdy notes in Aartsma-Rus 2004 (Dowdy Suppl. Reb. ¶¶ 95-96) illustrate the state of the art with respect to testing PMOs for exon skipping. For example, Aartsma-Rus notes the presence of PMOs in the cytoplasm but offers no solution to improve the "poor nuclear uptake" observed. Dowdy Suppl. Reb. ¶ 95.

72. The remaining pre-priority date reference Dr. Dowdy cites is Morita et al., Bioorg. Med. Chm 2003; 11:2211-26 ("Morita 2003"). Dowdy Suppl. Reb. ¶ 96. Dr. Dowdy previously noted that Morita 2003 discloses that PMOs had *lower* binding affinity compared to other chemistries. Dowdy Rebuttal ¶ 48. Now Dr. Dowdy claims that Morita 2003 discloses that "PMOs Watson-Crick base pair with *similar efficiency*" as 2'OMePS AONs to support this opinion that a POSA would

²² The original sentence in Aartsma-Rus 2004 is: "Further optimization of the morpholino itself and the leash required for EPEI transfection may thus increase the levels of exon 46 skipping." Aartsma-Rus 2004 at 1396.

have extrapolated target region binding and exon skipping to a corresponding PMO. Dowdy Suppl. Reb. ¶ 203 (emphasis added). Although base pairing “efficiency” is a vague term, it appears that Dr. Dowdy’s interpretation of the statement in Morita 2003 is inconsistent from one report to the other.

73. In accordance with my understanding, Aartsma-Rus 2004 reported lower apparent binding affinity for morpholinos compared to 2’-O-Me-PS. Aartsma-Rus 2004 at 1393. Another source that Dr. Dowdy cites is consistent with Aartsma-Rus 2004. *See*, Moulton 2006 at Table 4.30.1:

Table 4.30.1 RNA Binding Affinity of Various Oligo Types Ranked by Dissociation Temperature in Physiological Isotonic Buffers	
Affinity	Type of oligo
Strongest	RNA:RNA, PNA:RNA, 2’-O-methyl-RNA:RNA (all very similar)
Strong	Morpholino:RNA
Medium	DNA:RNA
Weakest	Phosphorothioate:RNA

74. That PMOs were known to have lower binding affinity than 2’-O-Me-PS AONs would have indicated to a POSA that PMOs need to be longer than 2’-O-Me-PS to have the same activity.²³ The ‘851 patent’s disclosure that the 25-mer 2’-O-Me-PS AON targeting H53A(+23+47) had “very faint skipping” would not have lead a POSA to believe that the corresponding morpholino version or even shorter morpholinos encompassed by the claims would have detectable exon skipping activity.

75. Dr. Dowdy’s criticism that I “ignore[d] the wealth of knowledge in the field of PMO therapeutics available to a POSA as of June 2005” (Dowdy Suppl. Reb. ¶ 197) is unfounded. I do not dispute that researchers had shown that cells could uptake PMOs and that PMOs were usable in antisense applications by June 2005. Sazani demonstrated this with two morpholinos (supplied by

²³ For example, drisapersen was a 20 base long 2’-O-Me-PS AON whereas eteplirsen is a 30 base long PMO.

Gene Tools) in HeLa cells. Sazani et al., Nucleic Acids Res 2001; 29(19: 3965-74 (“Sazani 2001”) at 3966; Dowdy Suppl. Reb. ¶ 197. I also do not dispute that scientists were exploring use of PMOs as potential therapeutics as of June 2005. Dowdy Suppl. Reb. ¶ 198; *see* Muntoni et al., Neuromuscul. Disord 2005; 15:45-57, at 452 (“some data on [morpholino] safety in the human are available”).

76. However, as of June 2005, PMOs were relatively new to the exon skipping field. Gebiski 2003 was the first to describe *in vivo* exon skipping results using a morpholino AON. Gebiski 2003 at 1808; *see also* Aartsma-Rus 2004 (the morpholino was produced by “[r]ecent developments in oligochemistry”); Wilton and Fletcher 2005 at 224 (characterizing PMO as a “novel chemistry”). As I noted in my Supplemental Report, the Gebiski 2003 authors (which included Drs. Wilton and Fletcher) expressly stated that “generalizations regarding the superiority of the morpholino over the 2OMe PS chemistry are purely speculative at this stage.” Wood Suppl. ¶ 54; Gebiski 2003 at 1808. Dr. Dowdy simply ignores Gebiski’s statement.

77. Dr. Dowdy’s citation of articles discussing Aartsma-Rus 2004 years after its publication (Dowdy Suppl. Rep. ¶¶ 199-200) does not change my opinion that a POSA in June 2005 would not have been able to draw generalized conclusions regarding the relative activity of a PMO based on results with a 2’-O-Me AON. Wood Suppl. ¶¶ 54-60. As mentioned above, the Aartsma-Rus 2004 authors themselves stated they could not distinguish between the potential causes of the low levels of exon skipping observed. Aartsma-Rus 2004 at 1396 (“the low levels of exon skipping *may* be the result of poor nuclear uptake rather than the low efficiency of the morpholino” and suggesting optimizing sequence and the leash “*may* increase levels of exon 46 skipping”) (emphasis added). Despite this possibility, the Aartsma-Rus 2004 authors nevertheless concluded that 2’-O-Me-PS AONs were “the most favorable... at least for targeted DMD exon 46 skipping.” *Id.* at Abstract.

78. Other references cited by Dr. Dowdy were not published as of June 28, 2005, and thus were not available to inform the knowledge and understanding of a POSA. Dowdy Suppl. Reb. ¶¶ 93 (citing Fletcher et al., J. Gene Med 2006; 8:207-216 (“Fletcher 2006”) and Dr. Hastings’ Supplemental Report), 156 (citing ¶ 93), 203-204 (citing Wilton 2005, Fletcher 2006, Adams et al., BMC Mol. Biol. 2007; 8:57 (“Adams 2007”) and Heemskerk et al., J Gene Med 2009; 11:257-266. Yet even these post-priority date articles do not support that researchers accepted as a general proposition that results are translatable from a 2’O-Me-PS AON to a corresponding PMO.

79. For example, the statement from Wilton 2005 (published after the priority date in December 2005 per Dr. Dowdy) and results reported in Fletcher 2006 are limited to “intramuscular injections in the mdx mouse model of muscular dystrophy.” Dowdy Suppl. Reb. ¶ 204; *see also* Wood Suppl. ¶ 56 (discussing Fletcher 2006). And the “perfect concordance” reported by Adams et al. in July 2007 based on unpublished data was similarly unknown in June 2005. *See* Dowdy Suppl. Reb. ¶ 204. Regardless, as discussed in my Supplemental Report, the vague and unsupported statement in Adams 2007 did not amount to a claim of general translatability across backbone chemistries. Wood Suppl. ¶ 58.

80. Arechavala-Gomez et al., Human Gene Ther. 2007; 18:798-810 (“Arechavala-Gomez 2007”), published in September 2007, is a publication co-authored by Dr. Wilton that describes the comparative and systematic analysis undertaken to validate the sequence and chemistry of the exon 51 AON selected to go forward into clinical trials in the UK.²⁴ Arechavala-Gomez 2007 at Abstract. The selection process was a collaborative effort between three major groups working on

²⁴ The selected AON became eteplirsen, the first FDA-approved exon skipping drug. It is now widely understood that eteplirsen has limited efficacy. Thus, even despite the Archevala-Gomez authors’ best efforts to “produce a reliable and coherent picture” and “select the best possible AO to be used in a clinical trial” the results in patients are very sub-optimal.

developing exon skipping therapies for DMD from the UK, Leiden University, and UWA. As part of that validation, the authors report that they tested a PMO version of a 2-OMe sequence that “had not been effective at achieving exon skipping” in part because “*when these experiments were designed it was not clear whether one could extrapolate results between sequences when different chemistries were used.*” Arechavala-Gomez 2007, at 805 (emphasis added).

81. This statement in Arechavala-Gomez 2007 squarely refutes Dr. Dowdy’s assertion that a POSA in June 2005 would have understood or expected that the exon skipping activity reported in the UWA Patents for 2’-O-Me-PS AONs would generally translate to the corresponding PMOs. Dowdy Suppl. Reb. ¶¶ 93, 104, 153, 156-157, 203-204. It supports my assertion (and recollection) that there was insufficient knowledge in the field as of June 2005 for a POSA to have that understanding or expectation; *see also* Wood Opening ¶ 89.

82. Accordingly, after reviewing the UWA Patents’ specification, a POSA would not have understood the inventors to have possessed or invented any *morpholino* AONs that they knew or reasonably expected would induce exon 53 skipping.

IV. RESERVATION OF RIGHTS AND TRIAL EXHIBITS

83. I reserve the right to amend or supplement my opinions once I review any new/additional information and/or other new/additional documents or information subsequently produced by Sarepta, UWA, or any other party, including via opening expert reports of Sarepta’s and UWA’s experts.

84. At trial I may use visual aids and demonstratives to show the bases for my opinions, such as photographs, drawings, excerpts from documents and materials I considered, videos, and animations. I reserve the right to utilize these at trial.

Exhibit 7

**IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE**

NIPPON SHINYAKU CO., LTD.,)

Plaintiff,)

v.)

SAREPTA THERAPEUTICS, INC.,)

Defendant.)

C.A. No. 21-1015 (JLH)

SAREPTA THERAPEUTICS, INC. and)
THE UNIVERSITY OF WESTERN)
AUSTRALIA, Defendant and Counter-)
Plaintiff)

v.)

NIPPON SHINYAKU CO., LTD. and)
NS PHARMA, INC., Plaintiff and)
Counter-Defendants.)

SUPPLEMENTAL EXPERT REPORT OF DR. MATTHEW J.A. WOOD

July 3, 2024



Matthew J.A. Wood, F.Med.Sci., MA, D.Phil.

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I. INTRODUCTION

1. I have been asked by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) to offer additional opinions in view of the Court’s clarification and/or amendment of its prior claim construction order. This report supplements the opinions expressed in my September 8, 2023 Expert Report (“Opening Report”), October 11, 2023 Expert Rebuttal Report (“Rebuttal Report”), and my October 27, 2023 Expert Reply Report (“Reply Report”) in view of the Court’s May 8, 2024 order regarding claim construction. A full list of materials I have considered in connection with this matter is attached as Exhibit 1, with the additional materials considered in connection with this Supplemental Report indicated in bold.

II. QUALIFICATIONS AND LEGAL STANDARDS

2. A discussion of my qualifications and compensation was provided in my Opening Report. My Opening Report also includes a discussion of the chain of priority of U.S. Patent No. 9,994,851 (“the ’851 patent”) and two other asserted University of Western Australia (“UWA”) patents, U.S. 10,227,590 and U.S. 10,266,827 (together with the ’851 patent, the “UWA Patents”).

3. I understand that, from the claims of the UWA Patents, Sarepta is now only asserting claim 1 of the ’851 patent against NS, therefore I have focused my analysis on that claim. However, my opinions are equally applicable to the other claims of the UWA Patents because they share a common specification, and the claims include many identical requirements.

4. I have been informed by counsel that the parties have agreed that a person of ordinary skill in the art (“POSA”) for the UWA Patents is an individual that has an M.D., Ph.D. or lower degree with expertise in molecular biology, biochemistry or a related area, and experience with neuromuscular or genetic diseases and/or designing and testing antisense oligonucleotides for splice-site switching/exon skipping applications. The POSA would have general knowledge of antisense

oligonucleotide chemical modifications to the backbone, nucleobases and other manipulations that can alter the activity of the antisense molecule, as well as delivery methods for antisense oligonucleotides. A POSA would also have general knowledge regarding using antisense oligonucleotides in cell-free, cell-based and/or in vivo experiments, as well as DMD models and the use of antisense oligonucleotides to induce skipping of DMD exons to correct the open reading frame of the RNA transcripts. I was a POSA as of 2005. I have rendered my opinions from the perspective of a POSA.

III. CLAIM CONSTRUCTION

5. Claim 1 of the '851 patent recites as follows:

An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

Id. at claim 1. Claim 2 recites as follows:

A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

Id. at claim 2.

6. The '590 Patent has two claims. Claim 1 recites as follows:

An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human

dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

Id. at claim 1. Claim 2 recites as follows:

A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

Id. at claim 2.

7. The '827 Patent has two claims. Claim 1 recites as follows:

A method for treating a patient with Duchenne muscular dystrophy (DMD) in need thereof who has a mutation of the DMD gene that is amenable to exon 53 skipping, comprising administering to the patient an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

Id. at claim 1. Claim 2 depends from claim 1 and recites: "The method of claim 1, wherein the antisense oligonucleotide is administered intravenously." *Id.* at claim 2.

8. A comparison of the claims of the UWA Patents is provided below:

Table 1: Exemplary Claim Comparison

'851 Patent, cl. 1	'590 Patent, cl. 1	'827 Patent, cl. 1
An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, <u>wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69)</u> , wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.	An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.	<u>A method for treating a patient with Duchenne muscular dystrophy (DMD) in need thereof who has a mutation of the DMD gene that is amenable to exon 53 skipping, comprising administering to the patient</u> an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

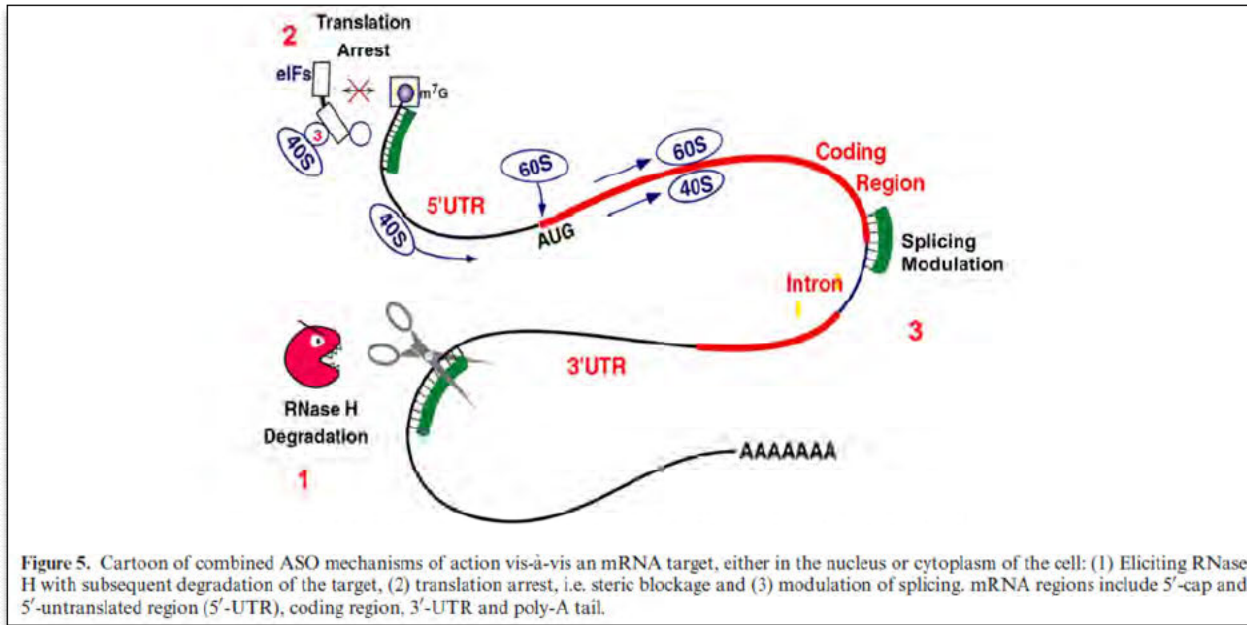
9. As discussed in my Opening Report, I have been informed by counsel that certain terms used in the claims of the UWA Patents have been construed by the Court, as shown in the table below:

Term #	Term	Claim	Court-Ordered Construction
[1]	“a base sequence”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “any sequence of bases that is part of the antisense oligonucleotide”
[2]	“a target region”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “a segment of the pre-mRNA”
[3]	“exon 53 of the human dystrophin pre-mRNA”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “the pre-mRNA transcribed from exon 53 of the human dystrophin gene”
[4]	“the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69)”	'851 Patent, claims 1 and 2	“the target region is within nucleotides +23 to +69 of exon 53 of the human dystrophin pre-mRNA”
[5]	“in which uracil bases are thymine bases”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	“the antisense oligonucleotide has thymine bases instead of uracil bases”

10. I further understand that the Court has clarified and/or amended in a May 8, 2024 oral order that claim 1 of the '851 patent “requires 100% complementarity to consecutive bases of a target region of exon 53 throughout the entire length of the antisense oligonucleotide.” I have applied these constructions, as clarified and/or amended, in my analysis. For terms that the Court determined should have their plain and ordinary meaning, I applied the meanings that would be understood by a POSA.

IV. EXON SKIPPING ACTIVITY IS UNPREDICTABLE

11. The precise mechanism by which antisense oligonucleotides (“AONs”) induce exon skipping has not been elucidated. A cartoon showing various AON mechanisms of action from Egli and Manoharan, *Nucleic Acids Res* 2023; 51(6):2529-73 (“Egli 2023”) is reproduced below, illustrating the complexities of the mechanism:



12. It is known, however, that exon skipping activity cannot be predicted based on the sequence of an AON alone. This is true even for AONs that are 100% complementary to the intended target region of the pre-mRNA throughout their entire lengths. Intrinsic factors of an AON, primarily sequence, length and backbone chemistry, affect exon skipping. As discussed in my Opening, Rebuttal and Reply Reports, even small changes to such parameters unpredictably affect activity. However, there are many variables extrinsic to the AON that unpredictably affect exon skipping activity.

A. Target Pre-mRNA Variables that Unpredictably Affect Exon Skipping

13. The interaction between an AON and the intended target region on the pre-mRNA is not just highly dependent on the sequence of nucleobases, but also highly dependent on proteins that bind the target sequence and their respective secondary and tertiary structures, as shown in the cartoon above. It remains difficult to predict the secondary structure of RNA. *See, e.g.,* Braasch and Corey, *Biochemistry* (2002); 41(14):4503-10 ("Braasch and Corey"); '851 patent, Col. 32:22-36; Egli 2023 at 2536.

14. The field of analyzing RNA structure (much less predicting that structure) remains in its infancy. The available data shows that: sequence-structure relationship of RNA under physiologically relevant solution conditions is highly complex and RNA structures are dynamic and a given sequence can adopt multiple structures. *See* Ding et al., Nat Commun. 2023; 14(1):714. RNA is therefore unlike proteins, such as antibodies, for which the premise that a primary sequence folds into a specific 3D structure under physiological conditions has generally been validated. RNA folding is “far more dynamic and heterogenous” and much more complex than protein folding. *Id.*

15. The inability to predict the 3D structure of mRNA is an important factor underlying the unpredictability of exon skipping. If the target region on the pre-mRNA is wholly inaccessible to the AON, it will be ineffective. However, because RNA folding is dynamic and heterogenous, whether a particular target region is accessible cannot be predicted and must be determined experimentally. This is particularly true in the field of AONs for dystrophin exon skipping.

16. In 2009, Popplewell et al., concluded that “no single design tool is likely to be sufficient in isolation to allow the design of a bioactive [AON], and empirical analysis is still required.” Popplewell et al., Mol Ther. 2009; 17(2):554-61 (“Popplewell 2009”) at 559. Popplewell looked at a number of factors including “the secondary structure of the pre-mRNA, which has a major role in determining [AON] bioactivity in cells.” *Id.* However, Popplewell concluded that a “hybridization analysis [is necessary] in determining what are likely to be the most bioactive oligomer.” *Id.* These results underscore that whether a particular target region is accessible could not be predicted—even after the publication of the UWA Patents. Moreover, Popplewell considered only secondary structure of the pre-mRNA—not its tertiary structure, which could significantly affect the binding of an AON to the target pre-mRNA.

17. Whether a target region of the pre-mRNA is implicated in the splicing process, and thereby more likely to be a good target for re-directing splicing, has been difficult to predict based on sequence. As of 2005, researchers studying dystrophin exon skipping had designed AONs targeting 3' and 5' splice sites, exon-internal sequences believed to be a splicing enhancer sequence (ESE), and purine-rich sequences resembling exon recognition sites. *See, e.g., Aartsma-Rus et al., Neuromuscular Disord* 2002; 12:S71-S77 (“Aartsma-Rus 2002”), at S72. However, the results were variable. As the UWA Patents' specification states, “[s]imply designing antisense molecules to target regions of pre-mRNA presumed to be involved in splicing is no guarantee of inducing efficient and specific exon skipping.” ’851 patent, Col. 4:32-34. Elsewhere in the specification the inventors stated that they have “discovered that there does not appear to be any standard motif that can be blocked or masked by antisense molecules to redirect splicing.” ’851 patent, Col. 24:3-6.

18. Although the approach of targeting regions “involved in mRNA splicing (i.e. splice donor sites, splice acceptor sites, or exonic splicing enhancer elements)... splicing branch points and exon recognition sequences or splice enhancers” (’851 patent, Col. 24:48-60) was conceptually reasonable, in practical terms, means of identifying such regions based on the mRNA sequence, were primitive and unreliable in 2005.

19. As stated in a 2007 publication from the Wilton laboratory: “[w]e found no definitive method to predict amenable sites within the pre-mRNA that, when targeted with an AO, would ensure efficient exon excision.” Harding et al., *Mol Ther* 2007; 15(1):157-166 (“Harding 2007”), at 161. The authors went on to describe how the software programs available at the time – Mfold and ESEfinder – were at best a starting point and recommended an empirical approach. *Id.* at 162-163, 164.

20. In cells, proteins bind to and alter pre-mRNA. Jankowsky & Harris, *Nat. Rev. Mol. Cell Biol.* 2016; 16(9):533-544 (“Jankowsky”) at 533. Accordingly, in addition to the tertiary structure of the pre-mRNA, protein binding affects AON binding and exon skipping activity. If the target region on the pre-mRNA is bound by a protein and the AON is therefore unable to bind, it will be ineffective. However, because of the complexity of RNA–protein interactions and the sheer number of proteins that can bind any given RNA molecule, whether a particular target region is bound by a protein and inaccessible to an AON cannot be predicted and must be determined experimentally.

21. In some circumstances, however, it may be beneficial to target a particular region that is known to bind splice factor proteins, such as SF2/ASF, SC34, SRp40, SRp55, Tra2 β , and 9G8. *See* Popplewell 2009 at 556. Targeting these regions could disrupt the RNA–protein interactions and therefore disrupt splicing. In 2005, Aartsma-Rus observed a “marginally significant” increase in effective AONs compared to ineffective AONs that targeted putative SF2/ASF and SC35 sites.¹ Aartsma-Rus et al., *Oligonucleotides* 2005; 15:284-297 (“Aartsma-Rus 2005”) at 292. Popplewell, however, found that there was a “significant association to PMO bioactivity” only with overlap for the predicted SF2/ASF binding site motif.² Popplewell 2009 at 559. Popplewell attributed the differences in results between the two studies to “AO chemistry used, [or] the number of AOs assessed.” *Id.* Moreover, Popplewell acknowledged that there is “weakness and unreliability in of SR protein–binding motifs as design tools for AOs” which “may be a reflection of the lack of precision of the predictive software used.” *Id.* Accordingly, it is still unpredictable how protein-binding sites in the mRNA target regions will affect AON activity.

¹ The data in Aartsma-Rus did not include AONs targeting exon 53. Rather, Aartsma-Rus looked at 77 AONs targeting exons 8, 17, 29, 43, 45-48, 51, 52, 54-63. Aartsma-Rus 2005 at 290.

² Popplewell’s conclusions are not limited to exon 53. Rather, Popplewell tested AONs targeting exons 44, 45, 46, 51, and 53. Popplewell 2009 at 559.

B. Factors Beyond the ASO and Target pre-mRNA Unpredictably Affect Exon Skipping

22. Even if RNA structure could be reliably predicted, it is widely understood that exon skipping function depends on an AON's intrinsic properties, *i.e.*, sequence, length, and backbone chemistry, **and** the 3D structure of the pre-mRNA (which is unknown and unpredictable) **and** a host of other variables which are not fully understood. Some of those variables, such as AON cell uptake, intracellular distribution, stability, and specificity, are affected by an AON's intrinsic properties. Others are fully extrinsic to the AON, such as method of delivery, cell type, and how exon skipping is measured. Each of these variables can affect whether an AON induces exon skipping or not.

23. For example, in Popplewell 2010, PMO-M, an AON targeting H53A(+39+69) did not induce detectable levels of exon skipping after intramuscular injection into humanized DMD mice, but did induce skipping when introduced by nucleofection into DMD patient cells. Popplewell et al., Neuromuscul Disord 2010; 20(2):102-10 ("Popplewell 2010"). There are many possible explanations for the differences in activity in these experiments. These include: differences in the secondary structures of the mouse and human pre-mRNA, methods of delivery and routes of administration, methods of cell uptake due to the different delivery methods, and cell type (mouse versus DMD patient cells). These potential explanations illustrate the many sources of variability extrinsic to the AON that affect evaluating exon skipping activity and make it highly unpredictable.

24. Further showing the influence of extrinsic factors on exon skipping activity, a 2019 publication from Dr. Wilton's group asserted that previously reported discrepancies in results obtained with AONs differing only in their backbone chemistry "arose from an issue of delivery rather than an ineffective AON, highlighting the need for confidence in efficient delivery in vitro during AON development." Aung-Htut et al., Molecules 2019; 24:2922-33 ("Aung-Htut 2019b"). The authors acknowledged the importance of efficient and effective delivery into cells: "[i]t is often

stated that there are three great challenges to genetic therapies: delivery, delivery and delivery.” *Id.* at 2928.

25. Because activity depends in part on factors extrinsic to the AON itself, it is not possible to predict whether an AON will induce dystrophin exon skipping based on its sequence and chemistry alone. Activity must be determined empirically, in properly controlled experiments. Both the UWA patent inventors and Sarepta acknowledged this principle applies to dystrophin exon skipping in the specification and during prosecution of various patent applications. *See, e.g.*, Opening Report ¶¶ 132-135, 145, 147; Rebuttal Report ¶¶ 70-79; Reply Report ¶¶ 6-14.

26. For the same reason, demonstrating that a set of AON structural features, *i.e.*, sequence, length, backbone chemistry, confers exon skipping activity would require extensive experimentation. That is assuming that any such structure-function relationship could be established at all given the many extrinsic variables that affect activity.

V. MORPHOLINO OLIGONUCLEOTIDES

27. As discussed in my Opening Report, phosphorodiamidate morpholino oligonucleotides (“PMOs”) have six-membered morpholine rings in place of ribose, and each nucleotide is joined by phosphorodiamidate linkages rather than phosphodiester linkages. Opening Report ¶ 61.

28. Morpholino oligonucleotides, which utilize a six-membered morpholine ring in place of ribose, were initially developed by James Summerton at Antivirals, Inc. Stirchak et al., *Nucleic Acid Res* 1989; 17(15):6129-41 (“Stirchak 1989”). Antivirals Inc. spun off Gene Tools LLC in 1997 for the manufacture and marketing of research quantities of morpholino oligos and later renamed itself AVI Biopharma Inc., which became Sarepta Therapeutics.³

³ https://www.gene-tools.com/about_us and https://www.gene-tools.com/history_production_and_properties

A. The State of the Art of Morpholino AONs as of 2005

29. As of 2005, AVI Biopharma had obtained compound and method of manufacturing patents, and its spin-off Gene Tools was the only commercial source of research quantities morpholino oligonucleotides worldwide for those looking to purchase them.⁴ Gene Tools offered morpholinos with four possible 3' end modifications in 2005 (3' primary amine (for conjugating additional moieties), a 3' lissamine, a 3' biotin and a 3' carboxyfluorescein) and began offering 5' modifications in 2006 by special arrangement.⁵ In terms of length, Gene Tools recommended 25-mers as their "longest available oligo" with up to 30-mers available for "a significant increase in price."⁶ However, even 25-mers were costly.⁷ I note that, because Gene Tools is and was only willing to synthesize oligos up to 30 bases in length, there is no commercial source for custom 31 base morpholino AONs.

30. Given the limited commercial options, a POSA looking to test a broader range of morpholino AONs would have to manufacture those AONs themselves. However, synthesizing and purifying morpholino AONs was not within the ability of most POSAs, requiring expertise in chemistry well beyond the definition set forth above. *See, e.g.*, Li et al., Nature Communications 2021; 12:4396 at 2-3 ("application of these potential advantages [of automated synthesizers] has been elusive"). There were also additional practical barriers to "in-house" synthesis. In 2005, only Gene Tools and AVI had access to morpholino phosphoroamidate monomers in anything less than bulk quantities. While a pharmaceutical company, such as NS, could purchase an industrial scale batch of monomer from a chemical supplier, that would be an enormous investment and not practical for any

⁴ <https://web.archive.org/web/20051220171348/http://www.gene-tools.com/node/9>.

⁵ <https://web.archive.org/web/20051222184949/http://www.gene-tools.com:80/node/23> and <https://web.archive.org/web/20060326092604/http://www.gene-tools.com/node/23>

⁶ <https://web.archive.org/web/20051220181301/http://www.gene-tools.com/node/5> and https://gene-tools.com/sites/default/files/Price_List_05May2024.pdf

⁷ https://web.archive.org/web/20060326093256/http://www.gene-tools.com/files/price_list.pdf

academic laboratory. Even today, research scale quantities of morpholino monomers are not commercially available due to stability issues.

31. Accordingly, I am not aware of any academic laboratory studying exon skipping AONs that has (or had) the capability to make morpholino AONs themselves, unlike other chemistries such as 2'-O-Me-PS. Thus, in my opinion, it would have been enormously challenging—if not impossible—for a POSA (as defined above) in 2005 to make and test any 31 base morpholino AONs, including those claimed by the UWA Patents. Similarly, it would have been difficult for a POSA to make and test morpholino AONs with other chemical modifications (*e.g.*, 5' or 3' modifications) beyond those offered by Gene Tools.

32. Assuming the AONs themselves were obtainable, neither a POSA nor team of POSAs would have been able to generate meaningful dystrophin exon skipping results in 2005 for a hundred or more AONs without a significant and essentially unachievable amount of experimentation. Researchers in the DMD field were only just beginning to experiment with morpholino AONs in 2005. And, while the difficulties of delivering morpholino AONs into cells were well-known, solutions to those difficulties and consistent techniques to assess dystrophin exon skipping *in vitro* and *in vivo* had not yet been established as of 2005.

33. Other limitations on the amount of experimentation a POSA was able to undertake include obtaining appropriate human cells, whether from normal subjects or DMD patients. The supply of such cells, particularly from DMD patients, is limited. Control and replicate experiments are needed to properly demonstrate exon skipping activity. Further, there is potentially significant variation in the normal human cells used to assess exon skipping assay from sample to sample. The passage numbers and growth conditions can also affect cell metabolism, which could affect uptake of AONs and gene expression—yet another source of unpredictability. Cells from DMD patients are

not only extremely challenging to obtain, but also vary significantly in ways that affect exon skipping experiments, such as where in the dystrophin gene mutations occur and disease progression.

1. Delivering morpholino AONs to cells was challenging

34. Difficulties with delivering morpholino AONs into cells and their nuclei was well-known as of June 2005. *See, e.g.*, Partridge et al., *Antisense Nucleic Acid Drug Dev* 1996; 6:169-175 (“Partridge 1996”); Summerton and Weller, *Antisense Nucleic Acid Drug Dev* 1997; 7:187-195 (“Summerton 1997”). Because morpholinos are neutral in charge, standard cationic transfection reagents cannot be used. *See, e.g.*, Gebiski et al., *Hum Mol Gen* 2003; 12(15): 1801-11 (“Gebiski 2003”).

35. Early techniques used to deliver naked morpholino oligomers into cells for *in vitro* assays, such as scrape-loading and electroporation, involved temporary physical or chemical disruption of the cell membrane. Morpholinos were also conjugated to cell-penetrating peptides to enhance their delivery. *See, e.g.*, Moulton and Yan, *Curr. Protoc. Mol. Biol.* 2008 (July); 83:26.8.1-29 (“Moulton and Yan”), at 26.8.17-18. Other approaches involved using high concentrations or extended incubation times to allow the morpholino to passively diffuse across the cell membrane. *See, e.g.*, Moulton and Yan, at 26.8.16. Gene Tools also developed a novel reagent, Endo-Porter, specifically for the purpose of delivering morpholino AONs and other non-ionic molecules into cells. Summerton, *Ann. N.Y. Acad. Sci.* 2005 (Nov); 1068:62-75.

36. Dr. Wilton’s laboratory developed an approach of annealing the morpholino AON to DNA or RNA molecules complementary to the morpholino, which they termed “leashes” in exon skipping assays.⁸ Gebiski 2003 at 1802. This created a charged molecule that could be used with standard cationic transfection reagents and allowed the leashed AON to be transfected into cells at

⁸ Much later, Dr. Wilton’s group reported that a leash was not necessary when particular transfection reagents were used. Aung-Htut 2019b.

lower concentrations than a naked morpholino. *Id.* at 1802. Some leashes included additional modifications with the goal of enhancing uptake of the AON, such as a cholesterol moiety. *Id.* at 1806.

37. Each of the foregoing attempts to develop methods for improving delivery of morpholinos varied in feasibility and effectiveness, and each would have introduced additional variables into any experiment. Further, variations within each approach (such as modifying leashes with additional moieties), would have been an additional source of unpredictability and experimental variability.

38. I note that despite the known difficulties with delivering morpholino AONs to cells, there is no guidance in the '851 patent specification regarding how to overcome such difficulties, much less a preferred approach. To the contrary, the specification's brief description of the experiments used to test AONs for exon skipping was understood *not* to work for morpholinos at the time. '851 patent, Col. 32:45-60 (describing transfection with "cationic lipoplexes, mixtures of antisense molecules or cationic liposome preparations").

2. There were only two reports on dystrophin exon skipping activity of morpholino AONs as of the priority date

39. Gebski 2003 was the first publication testing a morpholino AON to induce dystrophin exon skipping. It reports on testing of one morpholino AON targeting mouse exon 23 (M23D(+07-18), supplied by Gene Tools) that was annealed to various DNA/RNA leashes as well as the naked morpholino in *mdx* mouse cells *in vitro* and *mdx* mice *in vivo*. Gebski 2003. This morpholino was based on the sequence of an effective 2'-O-2'-Me-PS AON previously identified by the Wilton lab, extended by 5 bases per Gene Tools' recommendation that morpholinos should be at least 25 bases in length. *Id.* at 1809.

40. Gebski reported that the naked morpholino was ineffective, and the morpholino-leash AONs induced exon skipping to varying degrees *in vitro* in *mdx* mouse cells. *Id.* at 1803-04. One particular leashed AON was injected into *mdx* mice and induced dystrophin expression. *Id.* at 1806-07. The authors concluded that the particular morpholino tested can induce exon skipping when annealed to a leash and delivered as lipoplexes. *Id.* at 1809. No conclusions regarding a correlation of activity across backbone chemistries can be drawn because the morpholino tested had a different sequence and different length than the prior 2'-O-Me-PS AON, and was annealed to a leash.

41. In 2004, Judith Van Deutekom's group published a comparative analysis of AON analogs in an attempt to determine the best choice for future clinical studies. Aartsma-Rus et al., *Gene Ther* 2004; 11:1391-98 ("Aartsma-Rus 2004"). The AON analogs studied targeted exon 46, and included one morpholino, a 22-mer supplied by Gene Tools, linked to a leash of unspecified sequence. The article reports that in normal human myotubes, the morpholino AON had very low skipping activity (6%) compared to the corresponding 2'-O-Me-PS (20%) version. *Id.* at 1393-94. The relatively low level of activity was also shown in patient myotubes, with the morpholino inducing only 5% skipping compared to the 75% skipping observed with the 2'-O-Me-PS. *Id.* at 1394.

42. The authors offered multiple potential explanations for the low efficiency of the morpholino in both cell types, including lower affinity due to changes in secondary structure of the pre-mRNA, poor nuclear uptake, or the leash used. *Id.* at 1395-96. The authors noted that "for each different target sequence, the best choice of oligochemistry may vary." *Id.* at 1396.

43. Thus, as of June 28, 2005, there were only *two* publications describing the testing of morpholino AONs for inducing dystrophin exon skipping. One publication tested one morpholino AON and reported that morpholino AONs should be at least 25 bases in length, and the one tested induced mouse exon 23 skipping. Gebski 2003. The other publication, which was designed as a

comparative study, also tested one morpholino AON (a 22-mer) and reported that morpholinos were less effective than 2'-O-Me-PS, locked nucleic acid and peptide nucleic acid AON chemistries. A POSA would not be able to determine the source of the discrepancy because the two groups used different sequences targeting different exons, different cell types, different leashes, and different delivery methods. These differences underscore how those in the field were exploring various approaches to assessing morpholino AONs for exon skipping in cells *in vitro* and systemically *in vivo*.

3. Testing even ten morpholino AONs for dystrophin exon skipping would have been a significant and unprecedented effort

44. As of the June 28, 2005 filing date of the '851 Patent, researchers had tested a total of *two* morpholino AONs for dystrophin exon skipping activity. Testing even ten morpholino AONs for exon skipping would have been, at the time, completely unprecedented. A rigorous screen of hundreds of morpholino AONs for activity would have been far-fetched, if not impossible, in June 2005 due to limitations in both supply of and techniques for obtaining reproducible exon skipping results for this type of AON. As discussed below, even years after the filing date, researchers continued to test only a handful of morpholino AONs at a time.

45. In 2006, a multi-national group of researchers including Dr. Wilton reported that the systemic delivery of a PMO, M23D(+07-18), restored dystrophin expression in the *mdx* mouse. Alter et al., Nat Med. 2006; 12(2):174-7.

46. Also in 2006, Dr. Wilton's group published a paper on testing four PMOs total, unconjugated or conjugated to a peptide, targeting either C6A(+69+91) or C8A(-04+18). McClorey et al., Gene Ther 2006; 13:1373-1381. The authors concluded that poor cellular uptake was the cause of the "almost negligible exon skipping observed" with the PMO at all time points (1 to 10 days). *Id.*

at 1379. AVI Biopharma synthesized the PMOs and peptide-conjugated PMOs (“PPMOs”). *Id.* at 1380.

47. In 2007, Dr. Wilton’s group reported on the systemic distribution and intracellular uptake of one PPMO—M23D(+07-18), the same sequence they had previously tested in Gebiski 2003—in *mdx* mice. Fletcher et al., *Mol Ther* 2007; 15(9):1587-1592.

48. In January 2009, Dr. Van Deutekom’s group reported on an *in vivo* comparison of five 25-mer PMOs against corresponding 2’-O-Me-PS versions of varying length in *mdx* and (h)DMD mice. Heemskirk et al., *J Gene Med* 2009; 11:257-266, 258-59. The PMOs were obtained from Gene Tools. *Id.* at 258.

49. The first report from any laboratory on PMOs directed to exon 53 skipping was in January 2009, by Popplewell et al., *Mol Ther.* 2009; 17(2):554-61 (“Popplewell 2009”). As discussed in my Rebuttal Report, Popplewell designed a set of 66 PMOs to target exons 44-46, 51 and 53. Popplewell 2009 at 554. Of these, 23 targeted exon 53: 17 25-mers and 6 30-mers. *Id.* at Fig. 1F. The PMOs were synthesized by Gene Tools. *Id.* at 560. I note that the 30-mers tested by Popplewell was the maximum length PMO that Gene Tools was willing to synthesize in 2011, the same limit it currently imposes on length today.⁹ Popplewell published additional results on the exon skipping activity of the same exon 53 PMOs plus one additional in 2010. Popplewell 2010.

50. Also in 2010, AVI Biopharma’s Peter Sazani and Ryszard Kole published an international patent application, WO 2010/048586 (“Sazani ‘586”), reporting that they had synthesized and evaluated 24 overlapping peptide-conjugated PMOs targeting exon 53. Sazani ‘586 at 75. Sazani ‘586 also reports they synthesized and evaluated 26 peptide-conjugated PMOs targeting

⁹ <https://web.archive.org/web/20110526180010/http://www.gene-tools.com/node/15> and https://gene-tools.com/sites/default/files/Price_List_05May2024.pdf

exon 51, 17 targeting exon 50, 20 targeting exon 44, and 22 targeting exon 45, for a total of 109 AONs tested.

51. In 2011, Nippon Shinyaku submitted an international patent application reporting that they had tested 16 PMOs, one purchased from Gene Tools, the others synthesized in-house. *See* PCT/JP2011/070318, published as US 2013/0211062 (“Watanabe PCT ‘318”), at [0191] and Table 2. I note Watanabe PCT ‘318 disclosed PMOs with three different 5’ caps. *Id.* at [0162]-[163] and Table 2. The results reported by Watanabe for PMOs with the same sequence but different caps (PMO Nos. 3 and 13, and PMO Nos. 8 and 14), reflect how the 5’ cap can affect exon skipping activity. *Id.* at Fig. 8.

52. Thus, testing even ten morpholino AONs for dystrophin exon skipping was a significant effort that no one had undertaken as of June 28, 2005 or for years afterwards. Aside from Sazani, who likely was able to test 109 PMOs because AVI Biopharma synthesized them in-house, the greatest number of PMOs tested by a research group appeared to be 66: by Popplewell et al. in 2009. And there is no precedent for testing more than 24 morpholino AONs targeting exon 53 in a single study.

VI. A POSA WOULD NOT HAVE EXTRAPOLATED RESULTS ACROSS BACKBONE CHEMISTRIES

53. The backbone chemistry of the AON may not only confer specific chemical properties on the AON but may also affect its ability to access the target region. *See* Braasch and Corey at 4507. This is just one reason that, as of the June 28, 2005 filing date of the ‘851 patent, a POSA would not have had any expectations regarding the exon skipping activity of a PMO based on results obtained with a 2’-O-Me-PS with a similar or the even the same sequence, even if the AONs are 100% complementary to the target region of the pre-mRNA. There was no evidence to support such an extrapolation. Indeed, there were only two publications on using morpholino AONs to induce exon

skipping of the dystrophin gene as of the filing date: Gebski 2003 and Aartsma-Rus 2004. These two publications reported conflicting results regarding the efficiency of exon skipping by the morpholino AONs tested. *See, e.g.*, McClorey et al., Curr Op Pharmacol 2005; 5:529-534 (“McClorey 2005”), at 532 and Fletcher et al., J Gene Med 2006; 8:207-16 (“Fletcher 2006”), at 208 (both acknowledging the conflicting reports).

54. The paucity and inconsistency of the results in the literature underscores that using morpholino AONs for dystrophin exon skipping was in very early stages of development and that the relative activity of morpholino versus 2'-O-Me-PS AONs was unknown and could not be predicted or extrapolated, even if the AONs were 100% complementary to the target region of the pre-mRNA. *See, e.g.*, Gebski 2003 at 1808 (“generalizations regarding the superiority of the morpholino over the 2OMe PS chemistry are purely speculative at this stage.”). Even after the priority date, the inventors acknowledged that “many questions remain as to which AO chemistry and method of delivery will be most successful as treatment for DMD patients.” McClorey 2005 at 532. Thus, a POSA would not have assumed as a general matter that a morpholino and 2'-O-Me-PS AONs with the same or overlapping sequence would have similar exon skipping activity or that such activity could be extrapolated across backbone chemistries.

55. If anything, a POSA would have concluded based on Aartsma-Rus 2004, the only study designed to compare the relative efficacy of different backbone chemistries against the same target sequence on the pre-mRNA, which reported that morpholino AONs were *less* efficient than the corresponding 2'-O-Me-PS version. Thus, a POSA would not have assumed that a morpholino version of an ineffective 2'-O-Me-PS AON, such as SEQ ID NO: 195 of the '851 patent, would have detectable exon skipping activity.

56. Reflecting that the uncertainty and unpredictability concerning the effects of backbone chemistry continued after the filing date of the '851 patent, Dr. Wilton's group attempted to make a "direct comparison between the exon-skipping efficacy of the morpholino and 2'-O-Me-PS chemistries" in 2006. Fletcher 2006 at 208. This publication compared the activity of complexed (leashed) and uncomplexed morpholinos and 2'-O-Me-PS AOs targeting M23D(+07-18) *in vivo*. *Id.* The type of assay (*in vivo* vs. previous *in vitro* testing) and delivery method and regimen (intramuscular vs. single intraperitoneal vs. repeated intraperitoneal injections) used had dramatic effects on activity, illustrating how extrinsic factors beyond the intrinsic qualities of the AON affect exon skipping and render exon skipping highly unpredictable. *Id.* at 214.

57. Dr. Wilton's group continued work to establish that exon skipping results could be extrapolated from 2'-O-Me-PS to PMO AONs, showing that a POSA would not have made this extrapolation as of the priority date. It was not until 2007 that Dr. Wilton's group reported (based on unpublished data) "perfect concordance [has been] observed to date" in between the "exon skipping trends" of 41 2'-O-Me-PS previously shown to induce skipping and PMOs of the same sequence. Adams et al., BMC Mol Biol 2007; 8:57 ("Adams 2007"), at p. 6 of 8.

58. A POSA would not rely on a vague statement concerning "trends" based on unpublished data as sufficient basis to conclude that exon skipping activity of AONs could be universally extrapolated from 2'-O-Me-PS to PMO backbones. The authors do not make such an assertion, stating only that: "[d]etailed comparisons of sub-optimal PMOs to other dystrophin targets has not been undertaken, as the perfect concordance observed to date... would suggest that this would be unnecessary and a waste of resources." *Id.* Finally, that this publication came two years after the priority date reflects that Drs. Wilton and Fletcher had neither made nor recognized nor appreciated

that the reported exon skipping activity of SEQ ID NO: 195 or other “sub-optimal” sequences could be extrapolated to the PMO backbone chemistry as of the priority date.

59. The uncertainty in the field as to whether broad conclusions could be drawn regarding the relative efficiencies of the two backbone chemistries persisted after 2007. For example, it was not until 2009 that researchers reported the differences in efficiency between PMO and 2'-O-Me-PS observed for AONs targeting exon 23 in the *mdx* mouse were “less pronounced or even absent for human target sequences.” Heemskerk 2009 at 265. The authors also noted that PMOs may be less sequence-specific than 2'-O-Me-PS AONs, reflecting the unpredictable effects of backbone chemistry on exon skipping. *Id.* at 261-62, 265. However, the authors concluded “[d]ue to different experimental designs with regard to AON length, sequence, dose and injection frequency, it is difficult to draw conclusions on the relative efficiencies of 2OMePS and PMO AONs.” *Id.* at 258.

60. In view of the variable results from comparing the relative efficiency of morpholinos to other backbone chemistries, researchers from Leiden University hypothesized in 2007 that “it is likely that the optimal chemistry [for an AON] partly depends on the target sequence” based on reports comparing chemistries for AONs targeting exon 46, 51, 23, and others. Aartsma-Rus, RNA (2007); 13:1609-24, at 1619. This statement reflects the understanding in the field that the effects of backbone chemistry on skipping activity are unpredictable, and must be determined experimentally for individual AONs.

VII. THE GENUS OF COMPOUNDS ENCOMPASSED BY THE UWA PATENT CLAIMS

A. Post-Priority Date Evidence Reflects a Lack of Recognition or Appreciation of the Claimed Genus by Wilton and Others

61. My prior reports reflect my analysis of what a POSA would have understood the inventors of the UWA Patents to have invented after reviewing the specification. *See, e.g.*, Reply

Report ¶ 17. My Rebuttal and Reply Reports set forth support for my opinion that the UWA Patents do not disclose a “hot spot” within exon 53. Rebuttal Report, § IV.A and B.1; Reply Report § II.B.1.

62. It is further my opinion that a POSA would not have understood from the specification that the inventors recognized or appreciated a genus of exon 53 skipping compounds defined by the features recited in the claims of the UWA Patents, much less a relationship between that set of features and exon skipping activity. Nor would a POSA have understood the '851 patent to disclose such a genus or relationship. There is not enough information in the specification of the UWA Patents that would allow a POSA to draw any conclusions about whether the combination of sequence, length, and chemistry features recited in the claims, which allow for significant variation even when 100% complementary to the target region, generally confer exon skipping activity.

63. If there were such appreciation or recognition of such a genus by the inventors or those working in the field, their subsequent research would have focused on species within that genus to expeditiously advance a therapeutic AON for exon 53 given the urgent need for a DMD treatment and to efficiently use limited resources. However, post-priority date work by the inventors and others on exon 53 skipping AONs shows the opposite, with AONs that are *not* species of the claimed genus comprising the vast majority of those designed and tested than species within the claimed genus. This lack of focus demonstrates the lack of appreciation and recognition of the boundaries of the claimed genus or any purported relationship between the sequence, length, and chemistry recited in the claims and exon skipping activity.

64. The inventors reported on exon 53 skipping AONs in early 2007. Harding 2007. That publication does not reflect the testing of any species of the claimed genus. The inventors reported

testing one “pseudo-species”¹⁰ of claim 1 of the ’851 patent out of the 10 exon 53-targeting AONs tested. *Id.* at Table 1. That single pseudo-species was a 2’-O-Me-PS AON targeting +23+47, which was also SEQ ID NO: 195 in the UWA Patents.

65. The inventors described additional exon 53-targeting AONs in PCT Patent Publication No. WO 2011/057350 (“Wilton PCT ‘350”). Wilton PCT ‘350 discloses the inventors tested a total of 25 AONs targeting exon 53. Wilton PCT ‘350 at Table 43. None of these 25 AONs fall within the genus claimed by claim 1 of the ’851 Patent because none are morpholinos. As shown below, three AONs (targeting H53A(+27+56), H53A(+30+59), and H53A(+33+63)) are pseudo-species and the remaining 21 do not meet the length (L) or sequence limitations (S), or both (L/S).

¹⁰ I use the term “pseudo-species” to refer to an AON that meets the length and sequence limitations of claim 1 of the ’851 patent but is either not a morpholino with thymines instead of uracils or is inactive. Such AONs are not true species of the claimed genus because the claim requires: i) morpholino backbone chemistry; ii) thymines instead of uracils; and iii) exon 53 skipping activity. By way of example, SEQ ID NO: 195 is a pseudo-species because it is not a morpholino and has uracils, not thymines.

Summary of results reported in Wilton PCT '350

Pseudo-Species?	AON	Ability to Induce Skipping
No (S)	H53A(-15+15)	No skipping
No (S)	H53A(-32-06)	No skipping
No (S)	H53A(-38-13)	No skipping
No (S)	H53A(-49-26)	No skipping
No (S)	Hint52(-47-23)	No skipping
Yes	H53A(+27+56)	Strong skipping to 25 nM, faint at 5 nM
No (L)	H53A(+27+59)	Strong skipping to 10 nM, faint at 5 nM
Y*	H53A(+30+59)	Not reported
No (L)	H53A(+30+64)	Strong skipping to 25 nM, faint at 10 nM
No (L)	H53A(+30+69)	Strong skipping to 25 nM, faint at 5 nM
Yes	H53A(+33+63)	Strong skipping to 25 nM, faint at 5 nM
No (L)	H53A(+33+65)	Strong skipping to 25 nM, faint at 2.5 nM
No (L)	H53A(+33+67)	Strong skipping to 50 nM, faint at 5 nM
No (L)	H53A(+35+67)	Strong skipping to 25 nM
No (L)	H53A(+36+70)	Reasonable skipping to 5 nM
No (S)	H53A(+37+67)	Strong skipping to 25 nM
No (S)	H53A(+39+65)	Skipping 50 nM
No (S)	H53A(+39+67)	Skipping 100 nM
No (S)	H53A(+39+69)SNP	Skipping to 25 nM
No (S/L)	H53A(+39+71)	Strong skipping to 25 nM
No (S)	H53A(+40+70)	Skipping 50 nM
No (S)	H53A(+41+69)	Skipping 50 nM
No (S)	H53A(+42+71)	Strong skipping to 100 nM, faint at 5 nM
No (S)	H53A(+43+69)	Skipping 50 nM
No (S)	H53A(+69+98)	Skipping at 50 nM

S = does not meet sequence limitations; L = does not meet length limitations; S/L = meets neither sequence nor length limitations

* The exon skipping activity of one AON, H53A(+30+59), is not reported. The sequence is an AON published in Popplewell 2009, which was reported to have skipping activity.

66. Leaving backbone chemistry aside, as the table above shows, the large majority of the exon 53 AONs fail to meet the sequence and/or length limitations of claim 1. If the inventors had in fact previously identified a relationship between the structural sequence and length limitations recited in claim 1 and exon skipping activity, the rational approach would have been to design AONs having

those structural limitations. *See, e.g.*, Adams 2007 at p. 6 (suggesting that work with “sub-optimal” AONs would be “unnecessary and a waste of resources”).

67. As discussed above, in January 2009, George Dickson’s group at Royal Holloway University of London published Popplewell 2009, a paper reporting the design of PMOs to induce exon skipping in the DMD gene. Popplewell et al. designed the PMOs based on *in silico* predictions of exonic splicing enhancers. *Id.* at 555 and Fig. 1. Of the 23 leashed PMOs targeting exon 53, 4 were species and 19 were not species. *Id.* at Table S1.

68. In January 2010, Popplewell and others published another paper reporting the testing of the same 23 AONs for skipping exon 53, plus a PMO version of Dr. Wilton’s H53A(+39+69) as a comparator. Popplewell 2010. Popplewell 2010 used unleashed PMOs rather than the leashed PMOs tested in Popplewell 2009. Also, rather than the normal skeletal muscle cells used in the earlier study, Popplewell 2010 tested skipping in skeletal muscle cells from a DMD patient with a deletion of exons 45-52 and the humanized DMD mouse.¹¹ *Id.* at 103. However, Popplewell 2010 does not provide details on the cell number, density, or confluence, which are variables that could have affected the exon skipping activity of the AONs in patient cells. For example, using a defined number of cells is important as efficacy will relate to number of cells and concentration of the AON. This information is also important to enable other researchers to replicate Popplewell’s data.

69. A summary of the results reported in Popplewell 2009 and Popplewell 2010 is provided below.

¹¹ There are many types of patient cells that could have been selected for exon 53 skipping studies. This is another source of unpredictability. The humanized DMD mouse was engineered to express the full-length wild-type human dystrophin protein.

Summary of results reported in Popplewell 2009 and Popplewell 2010

Species?	Name (2009)	Name (2010)	Annealing Coordinates	Skipping hSKMC cells (2009)	Skipping Patient cells (2010)	Skipping hDMD mice (2010)
Y	h53A1	PMO-A	H53A(+35+59)	12.7%	68%	8%
N	h53A2	PMO-B	H53A(+38+62)	9.7%	56%	
N	h53A3	PMO-C	H53A(+41+65)	2%	15%-26%	
N	h53A4	PMO-D	H53A(+44+68)	10.5%	15%-26%	
N	h53A5	PMO-E	H53A(+47+71)	9%	15%-26%	
N	h53A6	PMO-F	H53A(+50+74)	0.3%	15%-26%	
Y	h53A30/1	PMO-G	H53A(+30+59)	52.4%	73%	7.2%
Y	h53A30/2	PMO-H	H53A(+33+62)	87.2%	68%	4.8%
Y	h53A30/3	PMO-I	H53A(+34+65)	80.1%	63%	7.6%
N	h53A30/4	PMO-J	H53A(+39+68)	38.6%	37%	
N	h53A30/5	PMO-K	H53A(+42+72)	9.4%	15%-26%	
N	h53A30/6	PMO-L	H53A(+45+74)	35.9%	15%-26%	
N	n/a	PMO-M	H53A(+39+69)		52%	<1% (limit of detection)
N	h53B1	PMO-N	H53A(+69+93)	0%		
N	h53B2	PMO-O	H53A(+80+104)	0.6%		
N	h53B3	PMO-P	H53A(+90+114)	3%		
N	h53C1	PMO-Q	H53A(+109+133)	0%		
N	h53C2	PMO-R	H53A(+116+140)	0%		
N	h53C3	PMO-S	H53A(+128+152)	0%		
N	h53D1	PMO-T	H53A(+149+173)	0%		
N	h53D2	PMO-U	H53A(+158+183)	0.1%		
N	h53D3	PMO-V	H53A(+170+194)	3.7%		
N	h53D4	PMO-W	H53A(+182+206)	12.3%		
N	h53D5	PMO-X	H53A(+188+212)	7.9%		

70. In my opinion, both Popplewell’s approach to designing the PMOs and the PMOs themselves illustrate how those working in the field neither recognized that Dr. Wilton had identified an exon 53 “hot spot” nor a correlation between the sequence limitations in claim 1 of the ‘851 patent and exon skipping activity. Further, Dr. Wilton is a co-author of Popplewell 2010, indicating he had some input into its contents. However, the paper does not acknowledge the prior discovery of a “hot spot” or amenable region of between +23 to +69 of exon 53. Instead, the authors reference a region

of +29 to +74 as being of interest, citing Popplewell 2009 and Wilton et al., Mol Ther 2007; 15:1288-96 (“Wilton 2007”).¹² *Id.* at 104.

71. Sazani ‘586, discussed above, tested one species of the claimed genus, a peptide-conjugated PMO targeting +23 to +47 designated SEQ ID NO: 429. Sazani ‘586 at 75. The remaining 23 exon 53-targeting AONs were not species. *Id.* From reviewing Sazani ‘586, a POSA would understand that the inventors had not relied on the prior disclosure of H53A(+23+47) by Dr. Wilton to design SEQ ID NO: 429; rather, the discussion in Sazani ‘586 implies that they discovered SEQ ID NO: 429 was identical after it was found to have activity. *Id.* at 76 (“when compared to other exon 53 antisense sequences, SEQ ID NO: 429 proved identical to H53A(+23+47) which is listed as SEQ ID NO: 195 in WO 2006/000057”). That only a single species was designed and tested—apparently by happenstance—reflects a lack of recognition of the claimed genus or any relationship between the recited structural features and exon skipping activity.

72. NS designed a series of 2’-O-Me-PS AONs to screen the entirety of exon 53 in 2009. Watanabe PCT ’318 at Table 7. NS conducted its screen of exon 53 over several experiments. The results of NS’s exon 53 AON screen is presented in the table below.

¹² Wilton 20007 discusses only one ASO targeting human exon 53: a 2’-O-Me-PS targeting h53A(+39+69), which is not a species within the claimed genus. Wilton 2007 at Table 1.

Summary of Results of NS's Exon 53 AON Screens

2'OMe AON	Length	% Skipping	2'OMe AON	Length	% Skipping	2'OMe AON	Length	% Skipping
H53A(+1+25)	25	0.0	H53A(+40+54)	15	4.8	H53A(+116+140)	25	0.0
H53A(+6+30)	25	0.0	H53A(+40+59)	20	41.9	H53A(+119+143)	25	0.0
H53A(+11+35)	25	22.4	H53A(+40+64)	25	53.2	H53A(+121+145)	25	26.9 ^a , 0.0 ^f
H53A(+16+40)	25	32.5	H53A(+41+58)	18	58.6	H53A(+124+148)	25	21.9
H53A(+21+45)	25	11.9	H53A(+41+65)	25	28.7 ^a , 28.0 ^e , 80.1 ⁱ	H53A(+126+150)	25	39.1 ^c , 0.0 ^e , 32.6 ^f
H53A(+26+50)	25	24.4	H53A(+42+61)	20	58.2	H53A(+128+152)	25	0.0
H53A(+29+53)	25	57.0	H53A(+44+61)	18	48.8	H53A(+131+155)	25	0.0
H53A(+30+54)	25	67.4	H53A(+45+59)	15	22.9	H53A(+136+160)	25	0.0
H53A(+31+55) *	25	42.2 ^a , 58.1 ^c , 74.5 ^b	H53A(+45+62)	18	52.6 ^b , 41.4 ⁱ	H53A(+141+165)	25	0.0
H53A(+32+49)	18	13.2	H53A(+46+70)	25	28.7	H53A(+144+168)	25	0.0
H53A(+32+51)	20	9.1	H53A(+51+75)	25	1.4	H53A(+146+170)	25	39.1 ^d , 0.0 ^e
H53A(+32+56) *	25	42.2 ^b , 58.1 ⁱ	H53A(+56+80)	25	1.6	H53A(+149+173)	25	0.0
H53A(+32+61)	30	91.0	H53A(+61+85)	25	2.7	H53A(+151+175)	25	13.3 ^d , 0.0 ^e , 11.5 ^e
H53A(+33+57) *	25	78.6 ^a , 95.1 ⁱ	H53A(+66+90)	25	2.7	H53A(+153+177)	25	0.0
H53A(+34+58) *	25	85.1 ^b , 94.8 ⁱ	H53A(+71+95)	25	4.2	H53A(+156+180)	25	0.0
H53A(+35+49)	15	7.5	H53A(+76+100)	25	3.2	H53A(+161+185)	25	0.0
H53A(+35+52)	18	26.4	H53A(+81+105)	25	6.4 ^b , 15.8 ^f	H53A(+166+190)	25	0.0
H53A(+35+54)	20	54.6	H53A(+84+108)	25	18.4	H53A(+171+195)	25	0.0
H53A(+35+59)	25	93.6 ^b , 93.1 ⁱ	H53A(+86+110)	25	0.0 ^b , 11.3 ^e , 13.9 ^f	H53A(+176+200)	25	0.0
H53A(+36+60)	25	73.1 ^a , 87.0 ^e , 87.2 ^b , 86.4 ⁱ	H53A(+88+112)	25	27.0	H53A(+179+203)	25	0.0
H53A(+37+56)	20	20.6	H53A(+91+115)	25	8.3 ^b , 9.7 ^f	H53A(+181+205)	25	1.1 ^d , 0.0 ^e
H53A(+37+61)	25	83.3 ^b , 84.1 ⁱ	H53A(+96+120)	25	0.0	H53A(+184+208)	25	0.0
H53A(+38+55)	18	23.8	H53A(+101+125)	25	0.0	H53A(+186+210)	25	1.8 ^d , 0.0 ^e , 16.4 ^e
H53A(+38+62)	25	51.6	H53A(+106+130)	25	0.0	H53A(+188+212)	25	0.0
H53A(+39+63)	25	47.2	H53A(+111+135)	25	0.0			

2'OMe AON	Length	% Skipping
H53A(+39+63)	25	40.8 ^a , 81.7 ^b , 46.1 ^c , 89.9 ^d , 64.1 ^e , 90.9 ^f , 100.0 ^g , 71.1 ^h , 76.2 ⁱ

^a Data represented in Fig. 9 of Watanabe PCT '318

^b Data represented in Fig. 10 of Watanabe PCT '318

^c Data represented in Fig. 11 of Watanabe PCT '318

^d Data represented in Fig. 12 of Watanabe PCT '318

^e Data represented in Fig. 13 of Watanabe PCT '318

^f Data represented in Fig. 14 of Watanabe PCT '318

^g Data represented in Fig. 15 of Watanabe PCT '318

^h Data represented in Fig. 16 of Watanabe PCT '318

ⁱ Data represented in Fig. 17 of Watanabe PCT '318

* made as a PMO

Watanabe PCT '318 at Figs. 9-17; NS000062121; NS00138319. Where a single AON was tested in more than one experiment, the results for each experiment are noted. Pseudo-species meeting the sequence, length and activity limitations are indicated in bold.

73. Notably, of the 74 2'-O-Me-PS AONs designed and tested by Watanabe et al., only 12 were pseudo-species (*i.e.*, meeting the sequence and length limitations of claim 1 in a 2'-O-Me-PS backbone). Again, that the NS inventors screened the entirety of exon 53 (despite being aware of Dr. Wilton's 2005 PCT application and other work) reflects a lack of recognition of the claimed genus and any relationship to exon skipping activity. Based on the results of their initial screen the NS inventors optimized position and length with a series of PMOs targeting the +31 to +57 region of exon 53 with different 5' end groups, along with PMO versions of prior art AONs. Watanabe et al., *Mol Ther Nucleic Acids* 2018; 13:442-449, at 443; *see also* Watanabe PCT '318 at Table 2. The published novel PMO sequences were all species of claim 1 of the '851 patent. Watanabe PCT '318 at Table 2 (PMO Nos. 1-10, 13-14).

74. In addition to the AONs they designed themselves, the NS inventors also used Dr. Wilton's H53A(+39+69) as a positive control in each experiment. Notably, the percentage of skipping observed with H53A(+39+69) varied significantly in each experiment. Other AONs that were tested more than once produced variable results. The variability in the results obtained with H53A(+39+69) underscore that factors not attributable to the AON or the pre-mRNA target will unpredictably affect exon skipping.

75. Finally, in one patent publication from Sarepta's Bestwick and Frank, WO 2014/100714, 9 exon 53 AONs were tested, of which 4 were species. *See* Example 1 (9 AONs tested; 4 species).

76. In sum, of the approximately 167 exon 53-targeting AONs that were designed, tested, and published by Dr. Wilton and other researchers discussed above, 16 were “pseudo-species” and only 21 were species of claim 1 of the ’851 patent. Of the 21 species, none were published by Dr. Wilton. Twelve were designed by the NS patent inventors after their initial screen of the entire exon. In my opinion, these numbers suggest that: 1) the inventors of the UWA Patents did not recognize or appreciate the parameters of the claimed genus when the UWA Patents were filed on June 28, 2005; and 2) the UWA Patents’ specification did not disclose to POSAs characteristics that eliminated the need for an empirical approach to designing exon 53 AONs.

B. The Genus Encompasses Many Structural Variations That Would Have Unpredictable Effects on Exon Skipping Activity

77. In addition to the variations in sequence and length, the UWA Patents state that the AONs of the invention could incorporate a range of additional chemical modifications, such as modified nucleobases, different internucleotide linkages, moieties and conjugates, or be part of chimeric or hybrid molecules, which increases the number of morpholino AONs encompassed by the claims of the UWA Patents. *See* ’851 patent, Col. 26:46-28:8; *see also* Opening Report ¶¶ 67-70.

78. The specification states that the invention encompasses oligonucleotides with nucleobase modifications or substitutions and provides nucleobases “particularly useful for increasing [] binding affinity” including “5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine” with “5-methylcytosine substitutions” as being “presently preferred base substitutions.” ’851 patent, col. 27:35-46.

79. Although it is possible to make morpholino AONs with the listed nucleobase modifications and substitutions (and the UWA Patents teach as much), I am not aware that any such AONs had been tested for exon skipping activity as of June 2005. Thus, the effect of each of these

modifications on exon skipping, including whether they would be useful for increasing binding affinity as stated in the specification, was unknown and unpredictable.

80. As discussed above, before June 2005, the Wilton laboratory was using leashes as a means of enhancing cellular uptake of morpholinos. *See* Gebski 2003. While the specification does not reference leashes as-such, use of such conjugates or moieties linked to the AON to enhance the cellular uptake of morpholinos was known in the art. *See* Gebski 2003; '851 patent, Col. 27:47-59. PMOs had also been modified with moieties to enhance their solubility. *See, e.g.*, US 2003/0166588 at [0049].

81. Other research groups developed PMOs conjugated to cell-penetrating peptides for the same purpose. Making such conjugates is not a trivial undertaking – each AON must be separately and independently conjugated to each peptide. Therefore, screening large numbers of conjugated morpholinos would have been a very difficult undertaking. Gene Tools also sells “Vivo-Morpholinos” comprising a morpholino oligomer covalently linked to an octa-guanidine dendrimer with or without further end modifications to achieve more efficient localized delivery.¹³

82. Finally, although in the antisense field the term “morpholino” is often used interchangeably with “PMO” (*see* Opening Report ¶ 58), inter-subunit linkages other than phosphorodiamidate have been and may be employed with the morpholine ring. *See, e.g.*, Stirchak 1989; U.S. Patent No. 5,185,444, Figs. 9-11; Summerton 1997 at 189 and Fig. 4 (describing use of carbonyl, sulfonyl, and phosphoryl linkages); Summerton, *Methods Mol Biol* 2017; 1565:1-15, at 11 (stating that Antivirals, Inc. made and tested many structural variations with a morpholine ring before going forward with the phosphorodiamidate linkage).

¹³ <https://www.gene-tools.com/vivomorpholinos>

83. The '851 patent claims an AON wherein the AON "is a morpholino antisense oligonucleotide." The '851 patent specification does not, however, define "morpholino." As discussed above, one of the first publications describing testing of a morpholino AON to induce dystrophin exon skipping came from Dr. Wilton's laboratory. That paper, Gebiski 2003, cited Summerton 1997. Figure 4 of the Summerton & Weller 1997 publication provides an example of the potential variation in inter subunit linkages of "morpholino" oligonucleotides:

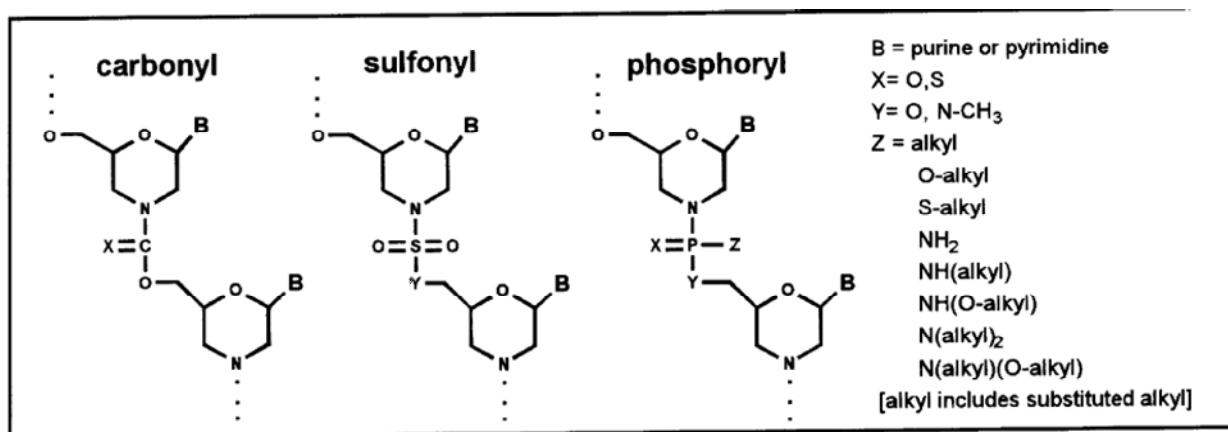


FIG. 4. Intersubunit linkage types for Morpholino oligos.

84. Thus, as of June 2005, POSAs were aware that "morpholino" AONs encompassed several types of inter-subunit linkages that each had many potential chemical variations. There is no indication in the '851 patent that the inventors intended the claimed "morpholino" to be limited to the phosphoryl type, much less PMOs specifically. If the term "morpholino" in claim 1 of the '851 patent is broader than PMOs, this would increase the number of different chemical structures encompassed by claim 1.

85. Even if "morpholino" is limited to phosphoryl linkages, there are extensive variations available, as illustrated in the Summerton 1997, Fig. 4, above. In that figure, there are 8 possible "Z" groups, and 2 possible groups for each of the X and Y, yielding a total of 32 combinations. This does not account for the indication in the figure that "alkyl includes substituted alkyl." Thus, a POSA

would have understood there were a large number of potential chemical structures encompassed by “morpholino” in claim 1 of the ’851 patent even if “morpholino” refers to PMOs.

86. In sum, the specification provides no information on how the myriad of structural variations and modifications encompassed by claim 1 affect exon skipping activity. A POSA is left to either speculate or make that determination independently, through trial-and-error experimentation.

VIII. RESERVATION OF RIGHTS AND TRIAL EXHIBITS

87. I reserve the right to amend or supplement my opinions once I review any new/additional information and/or other new/additional documents or information subsequently produced by Sarepta, UWA, or any other party, including via opening expert reports of Sarepta’s and UWA’s experts.

88. At trial I may use visual aids and demonstratives to show the bases for my opinions, such as photographs, drawings, excerpts from documents and materials I considered, videos, and animations. I reserve the right to utilize these at trial.

Exhibit 8

US006784291B2

(12) **United States Patent**
Iversen et al.

(10) **Patent No.:** **US 6,784,291 B2**
(45) **Date of Patent:** **Aug. 31, 2004**

(54) **SPLICE-REGION ANTISENSE
COMPOSITION AND METHOD**

- (75) Inventors: **Patrick L. Iversen**, Corvallis, OR
(US); **Robert Hudziak**, Blodgett, OR
(US)
- (73) Assignee: **AVI BioPharma, Inc.**, Corvallis, OR
(US)
- (*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 38 days.

(21) Appl. No.: **09/848,868**

(22) Filed: **May 4, 2001**

(65) **Prior Publication Data**

US 2003/0166588 A1 Sep. 4, 2003

Related U.S. Application Data

(60) Provisional application No. 60/202,376, filed on May 4,
2000.

(51) **Int. Cl.**⁷ **C07H 21/04**; C12Q 1/68;
C12P 19/34

(52) **U.S. Cl.** **536/24.5**; 536/24.3; 536/24.31;
536/24.33; 435/6; 435/91.1

(58) **Field of Search** 435/6, 91.1, 325,
435/375; 536/24.5, 24.3, 24.31, 24.33;
514/44

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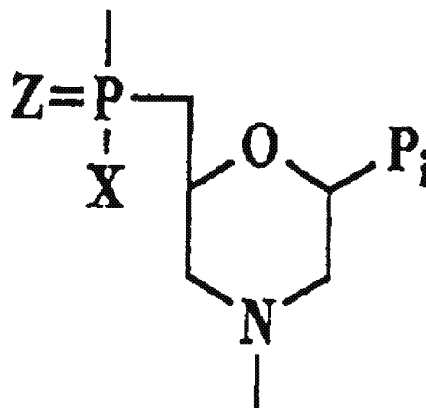
Primary Examiner—Karen A. Lacourciere

(74) *Attorney, Agent, or Firm*—LeeAnn Gorthey; Perkins
Coie LLP

(57) **ABSTRACT**

Antisense compositions targeted against an mRNA sequence
coding for a selected protein, at a region having its 5' end
from 1 to about 25 base pairs downstream of a normal splice
acceptor junction in the preprocessed mRNA, are disclosed.
The antisense compound is RNase-inactive, and is prefer-
ably a phosphorodiamidate-linked morpholino oligonucle-
otide. Such targeting is effective to inhibit natural mRNA
splice processing, produce splice variant mRNAs, and
inhibit normal expression of the protein.

6 Claims, 2 Drawing Sheets



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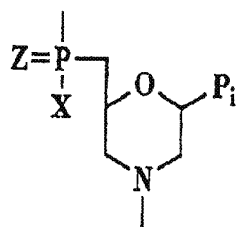


Fig. 1A

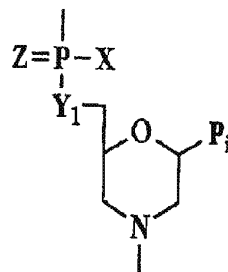


Fig. 1B

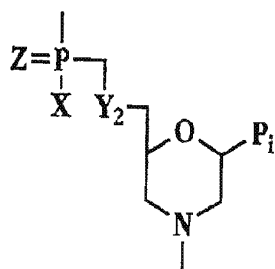


Fig. 1C

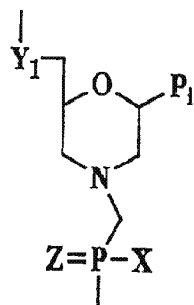


Fig. 1D

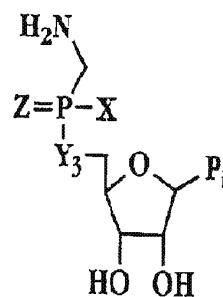


Fig. 1E

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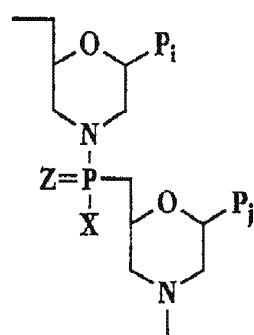


Fig. 2A-A

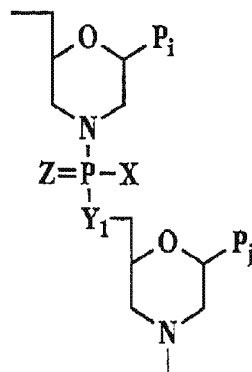


Fig. 2B-B

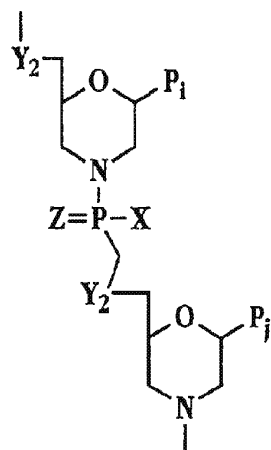


Fig. 2C-C

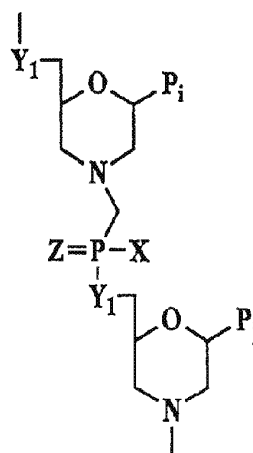


Fig. 2D-D/E-E

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SPLICE-REGION ANTISENSE COMPOSITION AND METHOD

This application claims priority to U.S. provisional application serial no. 60/202,376, filed May 4, 2000, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to therapeutic compositions and methods for inhibiting expression of full-length proteins in cells, and in particular to antisense compositions targeted against an mRNA sequence having its 5' end 1 to about 25 base pairs downstream of a normal splice acceptor junction in a preprocessed mRNA. Such targeting is effective to inhibit natural mRNA splice processing and produce splice variant mRNAs.

BACKGROUND OF THE INVENTION

Inhibition of protein expression by antisense targeting of DNA or RNA coding for the protein has been the subject of extensive study. Many reported procedures have employed phosphorothioate-linked oligonucleotides, which are charged, nuclease-resistant analogs of native DNA. The antisense mechanism involved is based on the activation of RNase, which cleaves the target nucleic acid to which the oligomer is bound. While these compounds have shown high activity, they also tend to show high levels of side effects, i.e. by cleavage of non-target RNA or by non-antisense mechanisms, such as nonspecific binding to proteins.

Another class of antisense oligomers, termed RNase-inactive, do not promote cleavage of bound RNA and are believed to act by sterically blocking the molecular machinery from transcribing, processing, or translating the target sequence. While these compounds tend to produce fewer side reactions, such as nonselective cleavage, than phosphorothioate oligomers, it has generally been necessary to target specific regions of RNA, such as the AUG start codon, for successful inhibition.

More recently, targeting of the splice acceptor junction of nuclear (unspliced) RNA by RNase-inactive oligomers has been reported. Krole and Dominski (U.S. Pat. No. 5,665,593) reported suppression of missplicing of β -globin RNA, in order to combat variants of β -thalassemia which result from such aberrant splicing. In this case, the aberrant splice junction was targeted, to direct splicing back to the normal site. R V Giles et al., *Antisense & Nucleic Acid Drug Dev.* 9:213-220 (1999), targeted a splice junction to induce missplicing of c-myc mRNA. In each of these cases, the region targeted is still somewhat restricted, in that the antisense oligomer spans the intron/exon splice junction of the pre-mRNA. Due to the advantages accorded by the use of uncharged, RNase-inactive oligonucleotides, a demonstration of further flexibility in targeting would be quite useful.

SUMMARY OF THE INVENTION

In one aspect, the invention provides an antisense compound, and a corresponding method of inhibiting normal splicing of preprocessed RNA in a eukaryotic cell, by contacting the cell with such an antisense compound. The compound is characterized by:

- (a1): an uncharged morpholino backbone;
- (a2): a base-sequence length of between 12 and 25 nucleotide bases; and
- (a3): a base sequence that is complementary to a target region of a selected preprocessed mRNA coding for a

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selected protein, where the 5' end of the target region is 1-25 bases downstream of a normal splice acceptor site in the preprocessed mRNA, and having the properties that:

- (b1): the compound is taken up by eukaryotic cells;
- (b2): the compound hybridizes to the target region of preprocessed mRNA in such cells, and
- (b3): the compound so hybridized to the target pre-mRNA prevents splicing at the normal acceptor splice site, such that the splice mechanism proceeds to a downstream splice acceptor site in the preprocessed mRNA, producing a splice variant processed mRNA with a truncated coding sequence.

In more specific embodiments, the 5' end of the target region is 2-20 bases, or 2-15 bases, downstream of the normal splice acceptor site. The length of the targeting compound is preferably about 15 to 20 nucleotide bases.

In one embodiment, the compound has intersubunit linkages selected from the group consisting of the structures presented in FIGS. 2AA-2EE. In preferred embodiments, the linkages are selected from a phosphorodiamidate linkage as represented at FIG. 2B-B, where $X=NH_2$, NHR , or NRR' , $Y=O$, and $Z=O$, and an alternate phosphorodiamidate linkage as represented at FIG. 2B-B, where $X=OR$, $Y=NH$ or NR , and $Z=O$. R and R' are groups which do not interfere with target binding. Preferably, R and R' are independently selected from alkyl and polyalkyleneoxy (e.g. PEG; $(CH_2CH_2O)_n$), or a combination thereof. The alkyl/polyalkyleneoxy chain may be substituted, preferably at the distal terminus, by a group selected from hydroxy, alkoxy, amino, alkylamino, thiol, alkanethiol, halogen, oxo, carboxylic acid, carboxylic ester, and inorganic ester (e.g. phosphate or sulfonate). Preferably, the chain (independent of substituents) is from 1 to 12 atoms long, and more preferably is from 1 to 6 atoms long. In selected embodiments, R and R' are independently methyl or ethyl. In one embodiment, $X=N(CH_3)_2$, $Y=O$, and $Z=O$.

NRR' may also represent a nitrogen heterocycle having 5-7 ring atoms selected from nitrogen, carbon, oxygen, and sulfur, and having at least as many carbon ring atoms as non-carbon ring atoms. Examples include morpholine, pyrrolidine, piperidine, pyridine, pyrimidine, pyrazine, triazine, triazole, pyrazole, pyrrole, isopyrrole, imidazole, oxazole, imidazole, isoxazole, and the like.

When the downstream splice acceptor site is a whole multiple of three bases downstream of the normal splice acceptor site, the splice variant mRNA has a coding sequence in frame with that of the processed mRNA when it is normally spliced.

The protein is preferably selected from the group consisting of myc, myb, rel, fos, jun, abl, bcl, p53, an integrin, a cathedrin, a telomerase, hCG, a receptor protein, a cytokine, a kinase, HIV rev, human papilloma virus, and human parvovirus B19. In selected embodiments, the protein is selected from myc, myb, abl, p53, hCG- β subunit, androgen receptor protein, and HIV-1 rev.

In further selected embodiments, the selected protein has multiple distinct binding regions, as in most transcription factors, and the truncated coding sequence codes for a variant protein in which one such binding region is disabled. Preferably, the variant protein is a dominant negative protein. One example is human c-myc, where the variant protein is an N-terminal truncated c-myc. In this embodiment, the antisense compound employed has a base sequence selected from the group consisting of SEQ ID NOs: 16 through 32 herein. The variant protein may also be a C-terminal altered c-myc, in which case the antisense

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compound employed can be an 18- to 20-mer having a base sequence which is a contiguous sequence selected from SEQ ID NO: 34; e.g. SEQ ID NO: 33.

In additional exemplary embodiments, the selected protein and the corresponding antisense base sequence(s) targeting its pre-mRNA are selected from the group consisting of:

- (a) human chorionic gonadotropin, β subunit: a contiguous 18- to 20-nucleotide sequence selected from SEQ ID NO: 15; e.g. SEQ ID NO: 14;
- (b) human androgen receptor: a contiguous 18- to 20-nucleotide sequence selected from SEQ ID NO: 9 or SEQ ID NO: 13; e.g. SEQ ID NO: 8 or 12, respectively;
- (c) human p53: a contiguous 18- to 20-nucleotide sequence selected from SEQ ID NO: 36; e.g. SEQ ID NO: 35;
- (d) human abl: a contiguous 18- to 20-nucleotide sequence selected from SEQ ID NO: 38; e.g. SEQ ID NO: 37; and
- (e) HIV-1 rev: a contiguous 18- to 20-nucleotide sequence selected from SEQ ID NO: 41; e.g. SEQ ID NO: 40.

These and other objects and features of the present invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows several preferred subunits having 5-atom (A), six-atom (B) and seven-atom (C-E) linking groups suitable for forming polymers; and

FIGS. 2A-A to 2E-E show the repeating subunit segment of exemplary morpholino oligonucleotides, designated A-A through E-E, constructed using subunits A-E, respectively, of FIG. 1.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The terms below, as used herein, have the following meanings, unless indicated otherwise:

"Antisense" refers to an oligomer having a sequence of nucleotide bases and a subunit-to-subunit backbone that allows the antisense oligomer to hybridize to a target sequence in an RNA by Watson-Crick base pairing, to form an RNA:oligomer heteroduplex within the target sequence, typically with an mRNA. The oligomer may have exact sequence complementarity to the target sequence or near complementarity. These antisense oligomers may block or inhibit translation of the mRNA, and/or modify the processing of an mRNA to produce a splice variant of the mRNA.

As used herein, the terms "compound", "agent", "oligomer" and "oligonucleotide" may be used interchangeably with respect to the antisense oligonucleotides of the invention.

As used herein, a "morpholino oligomer" refers to a polymeric molecule having a backbone which supports bases capable of hydrogen bonding to typical polynucleotides, wherein the polymer lacks a pentose sugar backbone moiety, and more specifically a ribose backbone linked by phosphodiester bonds which is typical of nucleotides and nucleosides, but instead contains a ring nitrogen with coupling through the ring nitrogen. Exemplary structures for antisense oligonucleotides for use in the invention include the morpholino subunit types shown in FIGS. 1A-E, with the linkages shown in FIGS. 2A-A to 2E-E. Such structures are described, for example, in Hudziak et al.,

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Antisense Nucleic Acid Drug Dev. 6, 267-272 (1996) and Summerton and Weller, *Antisense Nucleic Acid Drug Dev.* 7, 187-195 (1997).

Subunit A in FIG. 1 contains a 1-atom phosphorous-containing linkage which forms the five atom repeating-unit backbone shown at A-A in FIG. 2, where the morpholino rings are linked by a 1-atom phosphoamide linkage.

A preferred morpholino oligonucleotide is composed of morpholino subunit structures of the form shown in FIG. 2B-B, where the structures are linked together by phosphorodiamidate linkages, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, and Pi and Pj are purine or pyrimidine base-pairing moieties effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide. In preferred embodiments, the linkages are selected from a phosphorodiamidate linkage as represented at FIG. 2B-B, where X=NH₂, NHR, or NRR', Y=O, and Z=O, and an alternate phosphorodiamidate linkage as represented at FIG. 2B-B, where X=OR, Y=NH or NR, and Z=O. R and R' are groups which do not interfere with target binding. Preferably, R and R' are independently selected from alkyl and polyalkyleneoxy (e.g. PEG; (CH₂CH₂O)ⁿ), or a combination thereof. (An example of such a combination would be—(CH₂)₃(CH₂CH₂O)₃—). The alkyl/polyalkyleneoxy chain may be substituted, preferably at the distal terminus (i.e. the terminus not connected to the oligomer backbone), by a group selected from hydroxy, alkoxy, amino, alkylamino, thiol, alkanethiol, halogen, oxo, carboxylic acid, carboxylic ester, and inorganic ester (e.g. phosphate or sulfonate). Preferably, the chain (independent of substituents) is from 1 to 12 atoms long, and more preferably is from 1 to 6 atoms long. In selected embodiments, R and R' are independently methyl or ethyl. In one embodiment, X=N(CH₃)₂, Y=O, and Z=O. NRR' may also represent a nitrogen heterocycle having 5-7 ring atoms selected from nitrogen, carbon, oxygen, and sulfur, and having at least as many carbon ring atoms as non-carbon ring atoms. Examples include morpholine, pyrrolidine, piperidine, and pyridine.

Subunits C-E in FIG. 1 are designed for 7-atom unit-length backbones as shown for C-C through E-E in FIG. 2. In Structure C, the X moiety is as in Structure B and the moiety Y may be a methylene, sulfur, or preferably oxygen. In Structure D the X and Y moieties are as in Structure B. In Structure E, X is as in Structure B and Y is O, S, or NR. In all subunits depicted in FIGS. 1A-E, Z is O or S, and Pi or Pj is adenine, cytosine, guanine or uracil.

A "nuclease-resistant" oligomeric molecule (oligomer) is one whose backbone is not susceptible to nuclease cleavage.

As used herein, an oligonucleotide or antisense oligomer "specifically hybridizes" to a target polynucleotide if the oligomer hybridizes to the target under physiological conditions, with a T_m substantially greater than 37° C., preferably at least 50° C., and typically 60° C.-80° C. or higher. Such hybridization preferably corresponds to stringent hybridization conditions, selected to be about 10° C., and preferably about 50° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. At a given ionic strength and pH, the T_m is the temperature at which 50% of a target sequence hybridizes to a complementary polynucleotide.

Polynucleotides are described as "complementary" to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides. A double-stranded polynucleotide can be "complementary" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second.

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Complementarity (the degree that one polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules.

A "RNase-inactive" or "RNase-incompetent" oligonucleotide or oligonucleotide analog is one which acts via an RNase-independent mechanism, unlike RNase-active oligonucleotides, such as phosphorothioates. They are believed to function by sterically blocking target RNA formation, nucleocytoplasmic transport or translation, and are thus also referred to as "steric blockers". This class includes, for example, methylphosphonates, morpholino oligonucleotides, as described herein, peptide nucleic acids (PNA's), and 2'-O-allyl or 2'-O-alkyl modified oligonucleotides.

In a "peptide nucleic acid", the deoxyribose phosphate units of an oligonucleotide backbone are replaced with polyamide linkages. Proper backbone spacing is attained by the use of 2-aminoethyl glycine units, with a nucleotide base attached to each 2-amino group via a methylenecarbonyl group. A "2'-O-allyl (or alkyl) modified oligonucleotide" is an oligoribonucleotide in which the 2' hydroxyl is converted to an allyl or alkyl ether. The alkyl ether is typically a methyl ether.

"Alkyl" refers to a fully saturated acyclic monovalent radical containing carbon and hydrogen, which may be branched or a straight chain. Examples of alkyl groups are methyl, ethyl, n-butyl, t-butyl, n-heptyl, and isopropyl. "Lower alkyl" refers to an alkyl radical of one to six carbon atoms, and preferably one to four carbon atoms, as exemplified by methyl, ethyl, isopropyl, n-butyl, isobutyl, and t-butyl.

A "truncated" protein or coding sequence has some portion of the normal protein or sequence removed from one or the other terminus, from an internal region, or a combination of the above.

An amino-truncated (N-truncated) or carboxy-truncated (C-truncated) protein is one having an abnormal or deleted amine terminus or carboxy terminus, respectively, arising from translation of a splice variant mRNA.

II. Antisense Compounds

In accordance with the present invention, it has been discovered that an antisense compound having from 12 to 25 nucleotides, including a targeting base sequence that is complementary to a target region of a selected preprocessed mRNA coding for a selected protein, where the 5' end of the target region is 1 to 25 bases downstream, preferably 2 to 20 bases downstream, and more preferably 2 to 15 bases downstream, of a normal splice acceptor site in the preprocessed mRNA, is effective to inhibit splicing at the normal splice acceptor site and thus produce splice variant mRNA, leading to truncated or otherwise aberrant versions of the selected protein upon translation. Advantages of this strategy are set forth below.

The antisense compound employed in the present invention is one that does not activate RNase H. RNase-H active oligomers, of which phosphorothioate oligonucleotides are the most prominent example, operate primarily by a mechanism in which the target mRNA is cleaved. RNase-incompetent oligomers, on the other hand, are believed to act by a steric blocking mechanism. Such compounds include morpholino oligomers, PNA's (peptide nucleic acids), methylphosphonates, and 2'-O-alkyl or -allyl modified oligonucleotides, all of which are known in the art. The preferred antisense oligomers (compounds) of the present invention are morpholino oligomers, which are composed of

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morpholino subunits of the form shown in U.S. Pat. Nos. 5,698,685, 5,217,866, 5,142,047, 5,034,506, 5,166,315, 5,521,063, and 5,506,337, all of which are incorporated herein by reference. The synthesis, structures, and binding characteristics of morpholino oligomers are detailed in these patents. In a morpholino oligomer, (i) the morpholino groups are linked together by uncharged phosphorus-containing linkages, one to three atoms long, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, and (ii) the base attached to the morpholino group is a purine or pyrimidine base-pairing moiety effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide. The purine or pyrimidine base-pairing moiety is typically adenine, cytosine, guanine, uracil or thymine. Preparation of such oligomers is described in detail in U.S. Pat. No. 5,185,444 (Summerton and Weller, 1993), which is hereby incorporated by reference in its entirety. As shown in the reference, several types of nonionic linkages may be used to construct a morpholino backbone.

Such morpholino oligomers have shown high binding affinity for RNA targets, and the uncharged backbone favors uptake into cells and reduces non-specific binding interactions, relative to charged analogs such as phosphorothioates. They have been shown to provide significantly improved activity and selectivity in inhibiting translation of targeted sequences in comparison to phosphorothioate oligonucleotides. See, for example, Summerton et al., *Antisense & Nucleic Acid Drug Dev.* 7(2):63-70, Apr 1997. The morpholino oligomers have very high nuclease resistance and good water solubility, making them good candidates for in vivo use. Efficient uptake by cells in vivo is demonstrated in co-owned and copending application serial no. 09/493,427 and the corresponding PCT Pubn. No. WO 0044897. As described therein, morpholino oligonucleotides having phosphoramidate linkages formed heteroduplexes with target RNA, which are protected in this duplex state from nuclease degradation. Such a duplex is expelled from the cell, and the target RNA can later be detected in a body fluid sample from the subject. These results demonstrated that the morpholino oligomers (i) migrate to and enter cells in the body and (ii) bind with high affinity, via Watson-Crick base-pairing, to target nucleic acid regions.

Exemplary backbone structures for antisense oligonucleotides of the invention include the β -morpholino subunit types shown in FIG. 1A-E, as described above. It will be appreciated that a polynucleotide may contain more than one linkage type.

A preferred morpholino oligonucleotide is composed of morpholino subunit structures of the form shown in FIG. 2B-B, where the structures are linked together by phosphorodiamidate linkages, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, P_i and P_j are purine or pyrimidine base-pairing moieties effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide. In preferred embodiments, the linkages are selected from a phosphorodiamidate linkage as represented at FIG. 2B-B, where $X=NH_2$, NHR , or NRR' , $Y=O$, and $Z=O$, and an alternate phosphorodiamidate linkage as represented at FIG. 2B-B, where $X=OR$, $Y=NH$ or NR , and $Z=O$. R and R' are groups which do not interfere with target binding. Preferably, R and R' are independently selected from alkyl and polyalkyleneoxy (e.g. PEG; $(CH_2CH_2O)_n$), or a combination thereof. The alkyl/polyalkyleneoxy chain may be substituted, preferably at the distal terminus, by a group selected from hydroxy, alkoxy, amino, alkylamino, thiol, alkanethiol, halogen, oxo, carboxylic acid, carboxylic ester, and inorganic ester (e.g.

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phosphate or sulfonate). Preferably, the chain (independent of substituents) is from 1 to 12 atoms long, and more preferably is from 1 to 6 atoms long. In selected embodiments, R and R' are independently methyl or ethyl. In one embodiment, X=N(CH₃)₂, Y=O, and Z=O. NRR' may also represent a nitrogen heterocycle having 5–7 ring atoms selected from nitrogen, carbon, oxygen, and sulfur, and having at least as many carbon ring atoms as non-carbon ring atoms. Examples include morpholine, pyrrolidine, piperidine, pyridine, pyrimidine, pyrazine, triazine, triazole, pyrazole, pyrrole, isopyrrole, imidazole, oxazole, imidazole, isoxazole, and the like.

The solubility of the antisense compound, and the ability of the compound to resist precipitation on storage in solution, can be further enhanced by derivatizing the oligomer with a solubilizing moiety, such as a hydrophilic oligomer, or a charged moiety, such as a charged amino acid or organic acid. The moiety may be any biocompatible hydrophilic or charged moiety that can be coupled to the antisense compound and that does not interfere with compound binding to the target sequence. The moiety can be chemically attached to the antisense compound, e.g., at its 5' end, by well-known derivatization methods. One preferred moiety is a defined length oligo ethylene glycol moiety, such as triethyleneglycol, coupled covalently to the 5' end of the antisense compound through a carbonate linkage, via a piperazine linking group forming a carbamate linkage with triethyleneglycol, where the second piperazine nitrogen is coupled to the 5'-end phosphorodiamidate linkage of the antisense. Alternatively, or in addition, the compound may be designed to include one or a small number of charged backbone linkages, such as a phosphodiester linkage, preferably near one of the ends of the compound. The added moiety is preferably effective to enhance solubility of the compound to at least about 30 mg/ml, preferably at least 50 mg/ml in aqueous medium.

The compound is designed to hybridize to the target sequence under physiological conditions with a T_m substantially greater than 37° C., e.g., at least 50° C. and preferably 60° C–80° C. Although the compound is not necessarily 100% complementary to the target sequence, it is effective to stably and specifically bind to the target sequence such that expression of the target sequence, is modulated. The appropriate length of the oligomer to allow stable, effective binding combined with good specificity is about 8 to 40 nucleotide base units, and preferably about 12–25 base units. Mismatches, if present, are less destabilizing toward the end regions of the hybrid duplex than in the middle. Oligomer bases that allow degenerate base pairing with target bases are also contemplated, assuming base-pair specificity with the target is maintained.

III. Selection of Target Sequences

A. RNA Splicing: Background

The processing of nuclear RNA following transcription is observed in virtually all living cells. The mammalian genome contains genes that make transcripts of approximately 16,000 bases in length containing 7 to 8 exons. The process of splicing reduces the length of the mRNA to an average of 2,200 bases. The initial transcript is referred to as heterologous nuclear RNA (hnRNA) or pre-mRNA. Processing of hnRNA involves an aggregate of approximately 20 proteins, referred to collectively as the spliceosome, which carries out splicing and transport of mRNA from the nucleus. The spliceosome does not appear to scan from a common direction for all transcripts; introns may be removed in a reproducible order but not in a directional order. For example, introns 3 and 4 may be removed first,

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followed by removal of introns 2 and 5, followed by removal of introns 6. The order of intron removal is not predictable a priori of observation. The sequence recognition for processing is small, suggesting that errors or multiplicity of processing sites can be anticipated, and, in fact, as more genes are investigated, more variation in processing of hnRNA has been observed.

In preprocessed mRNA, the two-base sequence motifs at exon/intron junctions are invariant. The upstream (5') splice donor (SD) junction is of the form exon-/GT-intron, while the downstream (3') splice acceptor (SA) junction is of the form intron-AG/exon. The flanking bases are not invariant; however, the base immediately upstream of the splice acceptor AG sequence is C about 80% of the time.

The current understanding of intron sequence recognition is as follows:

5'-exonA₍₆₄₎G₍₇₃₎/G₍₁₀₀₎T₍₁₀₀₎A₍₆₂₎A₍₆₈₎G₍₈₄₎T₍₆₃₎...TAC-TAAC...C₍₈₀₎A₍₁₀₀₎G₍₁₀₀₎/exon-NN...-3'
splice donor (SD) splice acceptor (SA)

The numbers in parenthesis represent the approximate (when<100%) percent utilization of a base at a site. The bold A in the middle of the intron is the site (branch point) at which the G from the splice donor forms a branched 2'-5'-structure referred to as the lariat. The sequence indicated (TACTAAC) is the consensus observed in yeast. The consensus sequence in mammalian cells is PyNP₍₈₀₎Py₍₈₇₎Pu₍₇₅₎APY₍₇₅₎, where Py represents a pyrimidine (U/T or C) and Pu represents a purine (A or G). The A at the branch point is invariant, and is typically found about 12 to 50 bases upstream of the AG site. A pyrimidine-rich region (not shown above) is also generally found near the 3' end of the intron, about 10–15 bases upstream of the AG site.

Once the spliceosome forms the lariat, two transesterification reactions take place: 1) the 2'-OH of the branch point A in the intron to the 5'-phosphate of the SD intron G, and 2) the 3'-OH of the SD exon G to the 5'-phosphate of the first base of the SA exon. The removed intron is rapidly degraded in most cases, and the joined exons are now referred to as mature mRNA, which is transported out of the nucleus for translation into proteins by the ribosome.

B. Targeting Strategies

Various approaches could be taken to targeting the processing (splicing) of mRNA by antisense oligomers. The following sites could be targeted:

1. The exon-only portion of the SD exon (upstream of SD site), to interfere with SD processing.
2. The SD exon/intron junction, to interfere with SD processing.
3. The site of lariat formation within the intron, to block spliceosomal recognition upstream of the SA exon.
4. The SA intron/exon junction, to interfere with SA processing.
5. The exon-only portion of the SA exon (downstream of SA site), to interfere with SA processing.

Prior art methods have utilized strategy 2 or 4 (targeting of SD or SA junction). See, for example, RV Giles et al., cited above, in which a morpholino antisense oligomer spanning a splice acceptor site in the c-myc gene is described.

Experiments in support of the present invention found that targeting strategies 4 and 5 above, directed to the splice acceptor, were more reliably effective than strategies 1 and 2, directed to the splice donor.

For example, studies were carried out with rat CYP3A2 pre-mRNA targeted in vivo (whole animal). Animals were

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injected i.p. with 100 μ g PMO (as shown in FIG. 2B-B, where Y₁ and Z are oxygen and X is N(CH₃)₂) in phosphate buffered saline. The diminished rate of microsomal metabolism of erythromycin O-demethylase was monitored to reflect the expected phenotype caused by the antisense inhibition. As shown, the splice donor (SD) targeting was less effective than the splice acceptor (SA) strategy.

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using several proliferation assays. Primary fibroblasts from two species, rat and human, NRK and WI-38, respectively, were used for the screening.

Data shown in Table 1 employed NRK cells.

Data from [³H]thymidine incorporation assays using 20 μ M PMO (as shown in FIG. 2B-B, where Y₁ and Z are oxygen and X is N(CH₃)₂) are presented in Table 1. Per-

ANTISENSE SEQUENCE (/ indicates splice STRATEGY junction)		SEQ ID NO: ERDEM % of control
Control Saline	—	100 \pm 10.2 (N = 7)
SD 3'-AAGAGATGGC/CACTCACTGG-5'	4	94.7 \pm 5.4 (N = 3)
SA 3'-GGAAATATC/TGAACCTTGGG-5'	5	86.5 \pm 3.8 (N = 3)

Experiments with oligomers antisense to c-myc mRNA were conducted in cultured rat NRK cells, evaluating the inhibition of cellular DNA synthesis by monitoring incorporation of tritiated thymidine. Sequences were derived from Genbank Acc. No. Y00396 (rat) and J00120 (human), targeting the splice acceptor region at the beginning of exon 2 (with the exception of SEQ ID NO: 1, which targets the splice donor). The rat and human sequences indicated are highly homologous in this region. The oligomers listed in Table 1, below, were screened for antiproliferative effects

centages refer to [³H]thymidine incorporation relative to the vehicle (H₂O) control. Therefore, the lower the number, the greater the antiproliferative effect. It can be seen that all oligomers tested exhibited at least some antiproliferative activity. The extent of the inhibitory activity compared favorably with the antiproliferative drug Taxol (Paclitaxel, Bristol-Myers Squibb, Princeton, N.J.) at 32% of control. As 10%–20% of the cells are not affected by the scrape loading procedure and will, therefore, contribute to the residual [³H]thymidine incorporation activity, it is likely that most or all cells containing the most efficacious oligomers were growth inhibited.

TABLE 1

Cell Growth Inhibition by Anti-c-myc and Control Sequences		
SEQ ID NO: Antisense Sequence (5' \rightarrow 3')	Targeted Region*	Incorporation vs. vehicle control
1 CTGTGCTTAC/CGGGTTTCCACCTCCC (/ = SD site)	2553–2579	51 \pm 8%
2 ATCGTCGTGACTGT/CTGTTGGAGGG (/ = SA site)	4140–4164	27 \pm 3%
3 GCTCACGTTGAGGGGCATCG	4161–4180	38 \pm 2%
25 ACGTTGAGGGGCATCGTCGC	J00120 4515–34	29%
42 GGGCAUCGUCGUGACUGU/CUGUUGGAGGG	4140–4169	20%
43 CGUCGUGACUGU/CUGUUGGAGGG	4141–4162	45%
44 CGTCGTGACTGT/CTGTTGGAGG	4141–4162	21%
45 GGCAUCGUCGCGGGAGGCUG/CUGGAGCG	J00120 4498–4505	22%
46 CCGCGACAUAGGACGGAGAGCAGAGCCC	4364–4391	56%
47 ACTGTGAGGGCGATCGCTGC (scrambled)	—	~100%
48 ACGATGAGTGGCATACTGCG (3 mismatches)	—	>100%
49 CTCGCAATGCTGAAAGGTG (rat BCL-2)	—	>100%
50 GCGUGCCUCAAACAUGGUGGCGG (rat PCNA-1)	—	~100%

*Genbank Y00396 (rat) unless otherwise indicated

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Two irrelevant-sequence oligomers (rat BCL-2, SEQ ID NO: 49 and rat PCNA-1, SEQ ID NO: 50) did not inhibit NRK cells at the highest concentration assayed (20 μ M). mismatched and scrambled sequences of SEQ ID NO: 25 (SEQ ID NOs 48 and 47, respectively) had no effect on the proliferation of WI-38 (human) fibroblasts at 20 μ M.

SEQ ID NO: 45, which spans the 3'-splice acceptor site of the first intron of human myc pre-mRNA, was shown by Giles et al. (1999) to cause missplicing of myc pre-mRNA. A cryptic or latent splice acceptor, 44 bp distal to the normal splice acceptor position, was used to produce an aberrantly spliced mRNA with a 44-bp deletion. This misspliced mRNA lacks the initiator AUG and did not produce a normal myc protein.

SEQ ID NO: 25 does not span the intron 1-exon 2 boundary, the first potential complementary base pair being the tenth nucleotide from the 3' splice acceptor. Similarly, SEQ ID NO: 3 has its 5' end eleven bases downstream of the splice acceptor in the rat c-myc mRNA sequence. As the data shows, both sequences were effective in inhibiting cell growth.

It was of interest to determine if these oligomers inhibited normal splicing, as had been for SEQ ID NO: 45. Accordingly, RNA was prepared from human cells treated with positive (SEQ ID NO: 45) and negative (SEQ ID NO: 50) control oligomers as well as SEQ ID NO: 25. The structure of the RNA was then analyzed by making a DNA copy with reverse transcriptase and then performing PCR with flanking DNA primers (see Materials and Methods, below). The products of the RT-PCR procedure were analyzed by agarose gel electrophoresis.

In untreated or irrelevant oligomer-treated cells, a 304-bp band is predicted, and was observed, from the primers used and the c-myc nucleotide sequence. For cells treated with SEQ ID NO: 25, two DNA bands were observed. The upper band, which comigrated with the fragment from untreated or negative control cells, represented mRNA from the 10%–20% of cells not scrape loaded by the uptake procedure used, plus the fraction of correctly spliced mRNA in treated cells. The heavier lower band represented the misspliced, 44-bp depleted mRNA.

Cells loaded with SEQ ID NO: 25 also produced two bands from the RT-PCR procedure, one the size of normally spliced mRNA and one smaller. It can therefore be concluded that SEQ ID NO: 25, despite not directly overlapping the splice acceptor site, is capable of causing missplicing. The irrelevant PMO (SEQ ID NO: 50) gave an mRNA structure pattern identical to that of untreated cells, demonstrating that the missplicing is sequence specific.

A dose-response study with SEQ ID NO: 25 gave an IC_{50} of 3 μ M. The inhibitory effects began to plateau at 10 μ M, and there was little further change from 10 μ M to 20 μ M.

Myc protein has been implicated as important for the transition from G_0/G_1 of the cell cycle into the S phase (M K Mateyak et al., *Cell Growth Differ.* 8:1039–48, 1997). It could, therefore, be expected that if myc protein levels are reduced, the cells would arrest in G_1 . This effect was investigated by determining the number of cells in G_1 and G_2 , using the method of Telford et al. (*Cytometry* 13:137–43, 1993) (see Materials and Methods). After removal of RNA by hydrolysis, cells were stained by propidium iodide, a DNA-specific fluorescent dye. The DNA content per cell distribution was then determined by FACS analysis. The FACS intensity profiles show two peaks, corresponding to 2N DNA content (G_1) and 4N DNA (G_2). Cells treated with the PMO having SEQ ID NO: 25 showed an increase in the proportion of cells in G_1 (79% compared

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to 66% vehicle control) compared with those in G_2 (9% compared to 21% vehicle control). A positive control of quiescent cells obtained by growth factor starvation showed 80% of cells in G_1 and 8% in G_2 .

To obtain further evidence that the anti-c-myc oligomer SEQ ID NO: 25 inhibits c-myc mRNA expression by an antisense mechanism, a reporter gene model system was constructed to directly examine one gene (luciferase) and its activity (light production). The myc-luciferase reporter plasmid contained the entire 2.2-kb 5' region of human c-myc and the first 6 amino acid codons of the myc protein fused to the insect luciferase cDNA (see Hudziak et al., *Antisense Nucleic Acid Drug Dev.* 10:163–76 (2000)). It was transfected into HeLa cells, and a luciferase-producing clone was selected and designated clone L6. This cell line was scrape loaded with the indicated concentrations of PMO and replated. After 24–30 hours, the cells were lysed, and luciferase activity was measured (see Materials and Methods).

The results showed strong inhibition of luciferase production, with an IC_{50} of 300 nM. Several control experiments were performed to determine if the observed inhibition of luciferase synthesis was due to sequence-specific inhibition by the PMO. SEQ ID NO: 25 and the two sequence permutations noted above (SEQ ID NOs: 47 and 48) were compared.

Neither the scrambled version nor the 3-base mispair oligomer had any effect on luciferase production. Other control experiments showed that SEQ ID NO: 25 had no effect on HeLa proliferation during the 30-hour incubation time of the luciferase assay, and had no effect on an unrelated target reporter system (rabbit α -globin; J Summerton et al., *Antisense & Nucleic Acid Drug Dev.* 7:63–70, 1997) under conditions where a sequence complementary agent gave 70% inhibition. Other sequences containing 3–4 contiguous guanine (G) bases were tested in the NRK cell proliferation assay described above, and gave no significant inhibition of cell proliferation at concentrations up to 20 μ M.

In an evaluation of the “functional footprint” of PMO antisense directed to the splice acceptor (SA) region of rat c-myc, interference in splicing was observed with PMOs targeting the region from –44 upstream of the SA to +36 downstream (3' end of target region) from the SA. As the target sequence moved downstream, the proportion of lower molecular weight proteins, relative to normal c-myc, was observed to increase.

Targeting downstream of the splice acceptor junction, i.e. within the exon, is generally preferred, for reasons discussed below.

IV. Consequences of Interference with SA Site

If a binding oligomer interferes with normal mRNA splicing at the SA site, the spliceosome will proceed to the next best unblocked candidate site in the region. This site tends to be a [C]AG sequence with a short run of pyrimidines 10 to 15 bases upstream and a suitable branch point further upstream.

The resulting processed mRNA will generally be a splice variant mRNA in which the sequence between the normal splice acceptor site and the alternate (or “cryptic”) splice acceptor site has been deleted. The resulting variant protein can take different forms depending on the deleted sequence. For example, if the deleted sequence contains a whole multiple of three base pairs (that is, the downstream splice acceptor site is a whole multiple of three bases downstream of the normal splice acceptor site), the subsequent sequence will be in frame with the normal sequence, and a truncated form of the native protein will result. This permits the

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formation of dominant negative proteins, as described, for example, in Example C below. If the cryptic site is out of frame with the normal site, however, an unrelated “non-sense” protein will be produced. Due to the frequent occurrence of stop codons in non-reading frame sequences (about one per twenty codons), such a splice variant mRNA typically results in early termination of translation.

If the deleted sequence includes an AUG start site, translation may then occur at an alternate AUG site found further downstream. Again, if this alternate site is in frame with the normal AUG site, the resulting protein will have a deletion of some number of amino acids from the native protein. If the alternate site is out of frame, a “nonsense” protein, typically truncated early in translation, will result.

In one embodiment, exemplified in Examples A and B below, a carboxy-terminal truncated protein is created. In another embodiment, exemplified in Examples C and D below, a protein having an abnormal or deleted amino terminus is produced. The latter can be accomplished if the AUG translation start site is in exon 2 or greater of the mRNA.

Thus, when the sequence of pre-mRNA for a desired protein is known, an antisense target can be designed to alter desired regions of the protein. Preferably, the binding domains of the protein are also known, such that selected functions of the protein can be altered.

The following steps can be used to target genes with multiple exons, in accordance with the invention:

Step 1. Identify functional domains of the protein in question. The scientific literature can provide most of this information. One excellent source is “The Oncogene FactsBook” by Robin Hesketh, Academic Press, London, 1995, in which proteins related to cancer are reviewed and their functional domains mapped. Similarly, the “Cytokine FactsBook” (R E Callard and A J H Gearing, Academic Press) describes functional domains of cytokines. Other works in the same series include “The Protein Kinase Factsbook”, “The G-Protein Linked Receptor Factsbook”, and “The Extracellular Matrix Factsbook”.

Step 2. Search GenBank or similar nucleic acid databases for the gene sequence, including exon/intron sequences and junctions.

Step 3. Preferred antisense targets are within approximately 35–40 downstream bases of a splice acceptor site. Preferably, the target region has its 5' end at a location 1 to 25 bases downstream, more preferably 2 to 20 bases downstream, and most preferably 2 to 15 bases downstream, from the SA junction. (Note that a sequence one base downstream directly abuts, but does not overlap, the [C]AG splice acceptor site, and includes the first base of the normal exon.)

Step 4. To predict the consequence of inhibition, search the exon downstream of the SA for a cryptic splice acceptor, i.e. a [C]AG sequence downstream from the authentic SA. Once such a candidate site is located, search for a 5'-TACTAAC-3' (or similar) site for lariat formation 12 to 50 bases upstream of the cryptic SA. (Note that, according to the consensus sequence given above, only the branch point A must be conserved, so there is considerable flexibility in this sequence.) Preferably, there should also be a short pyrimidine-rich region 10–15 bases upstream of the cryptic SA.

Step 5. Once a potential cryptic SA site is identified, determine if its use will result in “in frame reading” by dividing the number of bases between the authentic site

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and the cryptic site by 3. If the quotient is a whole number, then the resultant protein will be “in frame” and possibly a dominant negative protein (see below).

Accordingly, in one preferred embodiment, an antisense target is chosen such that splicing is likely to be directed to a cryptic site whose use will result in in-frame reading.

V. Target Proteins and Selected Examples

Suitable target proteins include, for example, transcription factors, particularly oncogenic or proto-oncogenic proteins such as myc, myb, rel, fos, jun, abl, bcl, and p53; matrix proteins, such as integrins and cathegrins; other tumor-expressed proteins, such as hCG; telomerases; receptor proteins; cytokines; kinases; and viral proteins, such as HIV rev, human papilloma virus, and human parvovirus B 19. It is appreciated that inhibition of such proteins has numerous therapeutic applications. These include, but are not limited to, antitumor therapy, e.g. by targeting proteins, such as transcription factors, involved in various aspects of cell division and cell cycle regulation; antiviral or antibacterial therapy, by targeting proteins essential to replication or other vital functions of the infectious agent; and inhibition of restenosis or other proliferative disorders, by inhibiting proteins which support cell proliferation at the site.

Transcription factors are typically multidomain proteins, having a DNA binding region and a protein—protein binding region. Interfering with one of these regions can produce a dominant negative protein, which counters the activity of the native protein by preserving one activity (such as protein binding) while inhibiting another activity critical to the proper function of the protein (such as DNA binding; or vice versa). See the c-myc example described below.

As noted above, functional domains of many of the target proteins noted above have been studied extensively and reported in the literature. Sequences of pre-mRNA, including locations of introns, exons, and AUG start codons, can be found in the GenBank sequence database or other published sources readily available to those of skill in the art.

Following are several examples of antisense targeting downstream of splice acceptor domains in selected proteins to produce splice variants mRNAs which, upon translation, produce proteins with specific alterations.

In one embodiment, exemplified in Examples A and B, a carboxy-terminal truncated protein is created. In another embodiment, exemplified in Examples C and D, a protein having an abnormal or deleted amino terminus is produced.

A. Human Androgen Receptor (GenBank M35845, M35846)

A review of prostate cancer molecular biology indicates that androgen ablation is the state of the art therapy. In accordance with the present invention, this can be accomplished by inactivation of the androgen receptor. By targeting the center of the gene, at exon 2, various options are available for inhibiting expression of a functional androgen receptor. Proposed targeting of the splice acceptor in exon 2 (SEQ ID NO: 8; GenBank M35845) or exon 3 (SEQ ID NO: 11; GenBank M35846) is indicated.

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End of Intron 1: 5'- . . . TGTGTCTTTTCCAG/← splice acceptor site (SEQ ID NO: 6)
 Exon 2: 5'-TTTGAGACTGCCAGGGACCATG . . . -3' (SEQ ID NO: 7)
 Target antisense sequence: 5'-CATGGTCCCTGGCAGTCTCC-3' (SEQ ID NO: 8)

This oligomer (SEQ ID NO: 8) targets the sequence starting at base 48, four bases downstream of the normal splice acceptor site. The next probable cryptic splice site is at base 100 (CAG at 97-99; branch point A at 80; upstream pyrimidine region at 87-92. This site is out of frame with the normal site and should thus result in early termination of the protein.

Similar length oligomers targeting sequences having a 5' end 1 to about 18 bases downstream of the splice acceptor could also be used. This includes oligomers having a length of about 18 contiguous nucleotides selected from the sequence 5'-TCA ATG GGC AAA ACA TGG TCC CTG GCA GTC TCC AAA-3' (SEQ ID NO: 9; complementary to bases 45-80 of the sequence given in Genbank Acc. No. M35845).

End of intron 2: 5'- . . . TTTGTGTTCTCCAG/← splice acceptor site (SEQ ID NO: 10)
 Exon 3: 5'-GGAAACAGAAGTACCTGTGC GCC . . . -3' (SEQ ID NO: 11)
 Antisense sequence: 5'-GGC GCA CAG GTA CTT CTG-3' (SEQ ID NO: 12)

This oligomer (SEQ ID NO: 12) targets the sequence starting at base 49, six bases downstream of the normal splice acceptor site. A possible cryptic splice site is at base 145 (CAG at 143-145; branch point A at 114; pyrimidine region at 123-127). This site is in frame with the normal splice site and should thus result in a carboxy-truncated version of the native protein.

Similar length oligomers targeting sequences having a 5' end 1 to about 18 bases downstream of the splice acceptor could also be used. This includes, for example, oligomers having a length of about 18-20 contiguous nucleotides selected from the sequence 5'-AAT CAT TTC TGC TGG CGC ACA GGT ACT TCT GTT TCC-3' (SEQ ID NO: 13; complementary to bases 44-79 of the sequence given in Genbank Acc. No. M35846).

B. Human Chorionic Gonadotropin (β subunit) (GenBank X00266)

The β subunit of hCG is nearly identical to leutinizing hormone (LH) with the exception of the COOH end, which is extended in hCG. This extension is in exon 3 of the gene. By interfering with the exon 3 SA site, hCG could be truncated without targeting LH, unlike targeting of the AUG translation initiation sites, which are highly conserved in both proteins.

An oligomer with the sequence 5'-CCC CTG CAG CAC GCG GGT-3' (SEQ ID NO: 14) binds in exon 3 near (directly abutting) the SA (CAG at 1318-1320), targeting the sequence from bases 1321-1338, and interferes with splicing at this site. Similar length oligomers targeting sequences having a 5' end at base 1322, 1323, etc., up to about base 1340, could also be used. This includes, for example, oligomers having a length of about 18-20 contiguous nucleotides selected from the sequence 5'-GAG GCA GGG CCG GCA GGA CCC CCT GCA GCA CGC GGG T-3' (SEQ ID NO: 15; complementary to bases 1321-57 of the sequence given in Genbank Acc. No. X00266).

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Possible cryptic SA sites are at base 1393 (AG at 1391-2; branch point A at 1370 or 1373; in frame) and at 1458 (CAG at 1455-7; branch point A at 1427; out of frame). Splicing at either site would delete at least 24 amino acids from the protein, and possibly more, if the out of frame site were used. Since hCG is expressed only in tumor cells, this would be therapeutically beneficial in that less protein is expressed, and the protein will have a shorter biological half-life. In addition, the truncated protein may have unusual amino acids at the COOH end, possibly producing an immune response to hCG, useful in a vaccination strategy.

C. Human c-myc (GenBank J00120)

c-myc is a proto-oncogene which regulates cell growth and differentiation and is involved in the processes of vascular remodeling, smooth muscle cell proliferation,

extracellular matrix synthesis, and apoptosis. Aberrant expression of c-myc is frequently observed in human cancer. Aberrant, constitutive or overexpression of c-myc has been associated with a number of human cancers including lung cancer, colorectal cancer, breast cancer, bladder cancer, leukemia, lung cancer, etc. It has also been demonstrated that inhibition of c-myc reduces the incidence and severity of restenosis.

The c-myc protein has a DNA binding domain in the amine-terminal portion of the sequence and a protein—protein interacting domain in the carboxy-terminal portion. It is known that c-myc binds with Max in the carboxy domain to form a heterodimer that can bind to a DNA sequence known as an E-Box (5'-CACGTG-3'). When myc: max binds in this manner, the phenotype is growth stimulatory and can lead to apoptosis.

If myc concentration is low, then max forms homodimers which do not transactivate. If the mad protein is induced, then max binds mad to form mad: max heterodimers, which tend to induce differentiation and are anti-apoptotic.

Hence, simply inhibiting myc tends to result in the mad: max phenotype, which is anti-apoptotic. However, if the DNA binding (amine-terminal) domain of c-myc is inhibited, while leaving the protein—protein binding (carboxy-terminal) domain intact, the result is a dominant negative protein capable of binding max but not capable of transactivation. The favorable phenotype would be the loss of growth stimulatory actions, but the compensatory anti-apoptotic actions of mad: max would not dominate, as max protein can still bind to the myc COOH domain.

Inhibition of the amine-terminal domain is achieved by appropriate design of an antisense compound to direct alteration of splicing. Cryptic (alternate) SA sites found downstream include:

4547 Possible candidate; the next AUG, at 4554, is out of frame and would thus produce unrelated (“nonsense”) protein. (Could be blocked by oligos targeted further downstream than SEQ ID NO: 13)

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4578 Not a good SA candidate, due to few pyrimidines upstream
4617 A good SA candidate; next downstream AUG is at 4821, which is in frame with normal AUG at 4521

As described above, a morpholino oligomer (PMO) having a sequence complementary to bases 4515 to 4534 of the human c-myc mRNA sequence (Genbank Accession No. J00120), ten bases downstream of the splice acceptor site AG at 4504-5 (SEQ ID NO. 25, 5'-ACG TTG AGG GGC ATC GTC GC-3'), was found to prevent appropriate splicing at this site and the use of the normal AUG translation start site at 4521. Analysis of the mRNA produced indicated use of the 4617 site indicated above. Translation starting at the AUG at 4821, 300 bases downstream of the normal AUG, produces a protein having a 100 amino acid deletion at the N-terminus (as also reported by Giles et al.) Use of antibodies to the COOH end of myc revealed this protein, as well as substantially smaller proteins, from cells treated with the antisense oligomer. These N-terminal truncated proteins are expected to bind max but will not bind DNA.

These results, combined with examination of primer-amplified mRNAs, demonstrated the use of the 4617 cryptic splice site, as well as other cryptic sites further downstream.

As noted above, PMO oligomers targeted to the region from -44 upstream of the SA to +36 downstream (ds) from the SA showed evidence of interference with splicing in rat c-myc. Assuming an oligomer length of about 20 bases, the following antisense sequences could thus be utilized for targeting of human c-myc:

TABLE 2

Antisense Sequence	Bases ds of SA site SEQ ID (5' end of sequence) NO:	
5'-GGCATCGTCGCGGGAGGCTG-3'	1	16
5'-GGGCATCGTCGCGGGAGGCT-3'	2	17
5'-GGGGCATCGTCGCGGGAGGC-3'	3	18
5'-AGGGGCATCGTCGCGGGAGG-3'	4	19
5'-GAGGGGCATCGTCGCGGGAG-3'	5	20
5'-TGAGGGGCATCGTCGCGGGA-3'	6	21
5'-TTGAGGGGCATCGTCGCGGG-3'	7	22
5'-GTTGAGGGGCATCGTCGCGG-3'	8	23
5'-CGTTGAGGGGCATCGTCGCG-3'	9	24
5'-ACGTTGAGGGGCATCGTCGC-3'	10	25
5'-AACGTTGAGGGGCATCGTCG-3'	11	26
5'-TAACGTTGAGGGGCATCGTC-3'	12	27
5'-CTAACGTTGAGGGGCATCGT-3'	13	28
5'-GCTAACGTTGAGGGGCATCG-3'	14	29
5'-AGCTAACGTTGAGGGGCATC-3'	15	30
5'-AAGCTAACGTTGAGGGGCAT-3'	16	31
5'-GAGCTAACGTTGAGGGGCA-3'	17	32

The protein—protein binding (carboxy terminal) domain of myc could also be altered, as follows. An oligomer with the antisense sequence 5'-TCC TCA TCT TCT TGT TCC TC-3'(SEQ ID NO: 33) targets base 6656, downstream of

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the splice acceptor at base 6654-5. Likely downstream cryptic SA sites are at bases 6704, 6710, and 6729 (AG at 6702-3; CAG at 6707-09; CAG at 6726-8; A branch point at 6684; pyrimidine-rich region starting at 6690). Of these, the first two are out of frame and the third is in frame. Use of the third site would be expected to produce a deletion of 75 base pairs from the mRNA, resulting in a 25 amino acid deletion in the protein—protein binding domain of the myc protein. This protein would be the converse dominant negative, as DNA binding may be possible but no myc: max transactivation is likely. The function of myc would be lost, but the mad: max heterodimers would be favored, so that the phenotype of differentiation and anti-apoptosis would be observed.

As noted above, oligomers targeted progressively farther downstream, relative to SEQ ID NO: 33, could also be used. This includes, for example, oligomers having a length of about 18–20 contiguous nucleotides selected from the sequence 5'-AAC AAC ATC GAT TTC TTC CTC ATC TTC TTG TTC CTC-3' (SEQ ID NO: 34; complementary to bases 6656–91 of the sequence given in Genbank Acc. No. J00120). In accordance with the invention, an oligomer targeted far enough downstream to inhibit splicing at the first two cryptic sites noted above could be effective to promote splicing at the third cryptic site.

D. Human p53 (GenBank X54156)

Like c-myc, p53 has a non-coding exon 1, a large intron 1 and an AUG start codon near the SA site of exon 2. An oligomer targeted, for example, to the region having its 5' end at base 11691, three bases downstream of the SA site (5'-CCC GGA AGG CAG TCT GGC-3'; SEQ ID NO: 35) is expected to interfere with translation at the AUG initiation site as well as the normal splicing of exon 2. As described for c-myc above, other suitable oligomers include those targeted one or two bases downstream of the SA site, or targeted progressively further downstream, e.g. starting at base 11691, 11692, etc., and targeting some portion of the region between base 11689 (the first base of the normal exon 2) and about base 11725. This includes, for example, oligomers having a length of about 18–20 contiguous nucleotides selected from the sequence 5'-TCC TCC ATG GCA GTG ACC CGG AAG GCA GTC TGG CTG-3' (SEQ ID NO: 36; complementary to bases 11689–11724 of the sequence given in Genbank Acc. No. X54156).

Cryptic SA sites are available at base 11761 (AG at 11759-60) and at base 11765 (CAG at 11762-4) (A branch point at 11736; pyrimidine run at 11750-57). The next AUG, at base 11782, is out of frame, which will result in nonsense proteins. Alternatively, initiation may begin in exon 3, producing truncated p53-type proteins without the p53 amino terminus.

The following is an example in which targeting of the splice acceptor is especially advantageous.

E. Human Abl (GenBank AJ131466)

Since the bcr gene breaks and fuses to abl, forming the bcr-abl fusion protein, in chronic myeloid leukemia, it is a target of antisense inhibition. With respect to abl, fusion can occur at various locations; i.e. there are breaks after exon 1, 2 and 3 of bcr that fuse to Exon 2 of abl. However, by targeting the splice acceptor of abl, only one oligomer is required for treatment of all CML patients.

The bcr-abl fusion point is at the junction of bases 373–374. Therefore, the abl splice acceptor could be targeted by the following sequence, three bases downstream of this junction: 5'-CTA CTG GCC GCT GAAGGG C-3' (SEQ ID NO: 37).

Again, other oligomers targeting the region between the splice junction and about 35–40 bases downstream of the

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splice junction could also be used. This includes, for example, oligomers having a length of about 18–20 contiguous nucleotides selected from the sequence 5'-GCT CAA AGT CAG ATG CTA CTG GCC GCT GAA GGG CTT-3' (SEQ ID NO: 38; complementary to bases 374–409 of the sequence given in Genbank Acc. No. AJ131466). Possible cryptic splice sites (both out of frame) include CAG motifs at 468–70, with an upstream pyrimidine region at 453–459 and an A branch point at 421, and at 516–518, with an upstream pyrimidine region at 507–510 and an A branch point at 485.

F. HIV-1 (GenBank L39106)

This example illustrates a situation in which the method of the invention provides particular advantages; that is, where both a target virus and the host (human) express a gene with the same mRNA sequences. The protein products are important to the function of both virus and host but serve different functions.

An mRNA sequence of HIV-1 rev, which encodes a protein critical for viral replication 1 (see e.g. H Mitsuya et al., *Science* 249:1533–1543, 1990), was found to also occur in the host, leading to toxic effects when this sequence was inhibited by antisense. Specifically, a phosphorothioate oligomer having the sequence 5'-TCG TCG GTC TCT CCG CTT CTT CTT GCC-3' (SEQ ID NO: 39) was used to inhibit HIV-1 rev (Matsukura et al, *PNAS USA* 86:4244–4248, 1989). This region of rev is highly conserved in HIV; accordingly, it was targeted so that a large variety of viral isolates might be inhibited.

However, the preclinical development of this 27-mer was ended when 2 of 3 Rhesus monkeys treated by continuous infusion died of what appeared to be opportunistic infections. An excessive endotoxin burden was suspected, so the experiment was repeated, with care taken to remove endotoxin. However, two cynomolgous monkeys also died on days 8 and 9 of the continuous infusion in the repeat study. As endotoxin was effectively removed from consideration, immunosuppression was suggested as the cause. The white blood cell (WBC) count in the three Rhesus monkeys was 9.5 ± 0.7 prior to infusion with the antisense phosphorothioate to HIV-rev, and fell to 6.9 ± 0.6 during the infusion. There was no associated change in RBC or hematocrit. Further, surface marker studies for cells involved in immune response were influenced: CD2 was reduced from 88 to 76, CD8 fell from 45 to 36, and CD20 rose from 14 to 18.

A homologous region to HIV-rev observed in the genomes of humans and monkeys was reported in *J. Virology* 66:2170–2179 (1992), thus accounting for these toxic effects.

According to the present method, an alternative sequence could be selected which is less likely to interfere with host processes. In accordance with the present invention, HIV-rev could be suppressed as follows.

The protein is encoded by two exons: exon 1, 5493 . . . 5568, 76 bases; and exon 2, 7885 . . . 8180, 296 bases; 124 amino acids total (GenBank L39106). An antisense, RNase H-incompetent oligomer targeted to the region between the splice junction (i.e. the first base of normal exon 2) and about 35–40 bases downstream of the splice junction is expected to interfere with splicing of the pre-mRNA. As specified above, the 5' end of the targeted region is preferably 1 to about 25 bases downstream of the splice junction. An example of such an oligomer is a PMO targeted to base pairs 7885–7904, having the sequence 5'-CTC TGG TGG TGG GTAAGG GT-3' (SEQ ID NO: 40). Other candidates include oligomers having a length of about 18–20 contiguous nucleotides selected from the sequence 5'-CGG GTC

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TGT CGG GTT CCC TCT GGT GGT GGG TAA GGG T-3' (SEQ ID NO: 41; complementary to bases 7885–7921 of the sequence given in Genbank Acc. No. L39106).

The most likely site for a cryptic splice acceptor is at base 7975, where an AG sequence is preceded by multiple pyrimidines over 10 bases upstream from the AG. If this cryptic splice-acceptor is utilized, a deletion of 90 bases, or 30 amino acids, will result. This deletion will interfere with effective viral rev gene function.

VI. Analysis of Effects of Splice Acceptor Targeting

The effectiveness of a particular antisense sequence in producing splice variant mRNA may be determined by known analytical methods. For example, the presence or absence of the encoded, full-length protein and of truncated or other variant proteins can be monitored by standard techniques such as ELISA or Western blotting. Antibodies targeted to specific regions of the proteins, e.g. to the carboxy or amino terminus, can also be employed.

mRNA structure can be analyzed to evaluate antisense oligomer induced missplicing. The recovery of nuclear RNA is essential to observe intron-containing hnRNA, as the nucleus is the site of intron removal. Preparation of nuclear RNA is described in books such as "Molecular Cloning, A Laboratory Manual" (T. Maniatis, E. F. Fritsch and J. Sambrook, eds., Cold Spring Harbor Press) or "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., John Wiley & Sons, Inc.). The analysis of hnRNA is best done by either Northern blot or S1 Mapping. The presence of particular splice variant mature mRNAs can be determined by carrying out PCR amplification using selected primer pairs having sequences predicted to occur in a specific splice variant (or normal) processed mRNA. One primer is positioned in the SD (splice donor) exon upstream of the splice site, and the second is positioned in the SA (splice acceptor) exon, downstream of the splice site. Since the oligomer interferes with the SA site, the downstream primer should be more than 50 bases downstream from the SA splice site. Analysis of the PCR reactions on agarose gels stained with ethidium bromide will reveal amplified bands which are smaller in size than bands observed from untreated control cells, as evidence of blocking of the SA site by the oligomer.

VII. Advantages of the Method

The present invention demonstrates that inhibition or alteration of protein expression can be achieved by antisense targeting of a region downstream of a splice acceptor, in the coding region of a pre-mRNA, using a non-RNase competent oligomer. The present compositions and methods have several advantages over prior art methods of antisense inhibition, in which the targeting antisense compound spans and hybridizes to a splice acceptor sequence (e.g. Giles, cited above; Kole and Dominski, U.S. Pat. No. 5,665,593).

One such advantage is that exon sequences tend to much more highly conserved among species than intron sequences. This allows for greater predictability in testing such methods on animal models.

In addition, greater flexibility is allowed in selecting a sequence for targeting, as the oligomer is not required to actually bind to a precise site such as a splice acceptor junction or an AUG start codon. The present invention thus increases the range of sequences which may be successfully targeted in an antisense application. Such flexibility can be advantageous in avoiding undesirable side reactions, such as caused by inadvertent targeting of non-target proteins in a subject, or targeting of host proteins when attacking an infectious agent such as a virus or bacterium. This is demonstrated in Example F, above.

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In the present strategy, in contrast to methods which target the splice junction directly, various regions of the exon downstream of the SA site may be blocked. This opens the possibility of producing different variant proteins, by directing splicing to different cryptic splice sites downstream of the normal SA. As described above, certain variant proteins, such as dominant negative proteins, can have unique advantages. Accordingly, the antisense could be designed to promote splicing at a particular cryptic splice site, which would give rise to the desired variant protein, over others. For example, a less desirable cryptic splice site close to the normal SA site could be hindered in favor of a site further downstream.

Further benefits of flexibility of design may include convenience of synthesis or enhanced binding affinity. In addition, by using the present strategy of targeting fully within the exon, the target RNA can be identified by the antisense oligomer in either the nucleus or the cytoplasm, which can be advantageous for purposes of analysis.

VIII. Treatment Methods

In a related aspect, the invention includes a method of treating or preventing a disease state by inhibiting or altering expression of a target protein. Such disease states include viral, bacterial or fungal infections, cancerous tumors, and other conditions characterized by cellular proliferation, such as restenosis, hyperproliferative skin disorders, or inflammation. Proteins targeted, as noted above, include transcription factors, which include many oncogenes, receptor proteins, matrix proteins, and viral proteins. Inhibition of such proteins generally results in disruption of the cell cycle, viral replication, or other critical functions.

The method is carried out by administering to the subject an antisense oligomer 12 to 25 nucleotides in length and having (i) a base sequence complementary to a target region of a selected preprocessed mRNA coding for the target protein, where the 5' end of the target region is 1–25 bases downstream, and preferably 2–15 bases downstream, of a normal splice acceptor site in the preprocessed mRNA, and (ii) an uncharged morpholino backbone, preferably a phosphorodiamidate backbone as shown in FIG. 2B-B, where X, Y, and Z are as defined above. Preferably, the compound also contains a moiety that enhances the solubility of the compound, preferably to a solubility in aqueous medium of between 25–50 mg/ml or greater. An example is a polyethylene glycol (PEG) chain.

In general, the method comprises administering to a subject, in a suitable pharmaceutical carrier, an amount of the antisense agent effective to interfere with splicing at the normal splice acceptor site, and thus suppress normal expression of the protein. In a preferred embodiment, the method results in expression of a dominant negative variant of the protein. In one aspect of the method, the subject is a human subject.

Effective delivery of the antisense oligomer to the target mRNA is an important aspect of the method. PMOs have been shown to enter cells efficiently (see e.g. J Summerton et al., *Antisense Nucleic Acid Drug Dev.* 7:63–70, 1997, and copending and co-owned U.S. provisional application 60/117,846). For use in antiviral treatment, various systemic routes of delivery, including oral and parenteral routes, e.g., intravenous, subcutaneous, intraperitoneal, and intramuscular, as well as inhalation, transdermal and topical delivery, can be used.

Typically, one or more doses of antisense oligomer are administered, generally at regular intervals for a period of about one to two weeks. Preferred doses for oral administration are from about 1 mg oligomer/patient to about 25 mg

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oligomer/patient (based on a weight of 70 kg). In some cases, doses of greater than 25 mg oligomer/patient may be necessary. For IV administration, the preferred doses are from about 0.5 mg oligomer/patient to about 10 mg oligomer/patient (based on an adult weight of 70 kg). Dosages will vary in accordance with such factors as the age, health, sex, size and weight of the patient, the route of administration, and the efficacy of the oligonucleotide agent with respect to the particular disease state. For treatment of infectious agents, a preferred dosage is typically that which is necessary to attain a concentration in the blood of from about 0.01 to about 1 μ M, and more preferably about 200–400 nM antisense oligomer. This concentration can be achieved in a variety of ways; doses of between about 0.05 and about 0.2 mg/kg/hour by continuous IV infusion have been found to be acceptable. Greater or lesser amounts of oligonucleotide may be administered as required.

For treatment of hyperproliferative skin disorders, topical administration is indicated. In treatment of restenosis, delivery of the antisense oligomer to the affected cells, that is, to the site of arterial injury, is recommended. Delivery methods known in the field, such as those described in co-owned and copending U.S. application serial no. 09/493,427, can be used to deliver the oligomer to the site of angioplasty in a patient. Preferably, the oligomer is delivered concurrent with the angioplasty procedure. For an adult human, a recommended dosage is in the range of 1–25 μ mol of antisense oligomer, and preferably 2–15 μ mol. With respect to the surface area of tissue to be treated, an effective dose is typically in the range of 30 to 3000 μ g oligomer per cm^2 of vessel wall, and more preferably about 300 to 1500 μ g/ cm^2 . The patient may also be given the composition on a periodic basis after angioplasty, at a dosage level sufficient to further inhibit restenosis.

An effective in vivo treatment regimen using the antisense oligonucleotides of the invention will vary according to the frequency and route of administration, as well as the condition of the subject under treatment. Optimum dosages for a given route can be determined by routine experimentation according to methods known in the art. Such in vivo therapy is generally monitored by tests appropriate to the particular type of ailment being treated, and a corresponding adjustment in the dose or treatment regimen can be made in order to achieve an optimal therapeutic outcome.

Entry of a morpholino oligomer into cells and binding to its target RNA sequence can be verified by techniques set forth in copending and co-owned U.S. provisional application 60/117,846, which is incorporated herein by reference. A morpholino antisense compound of the type disclosed herein, when administered in vivo, can be detected in the urine of the receiving subject in a heteroduplex form consisting of the antisense compound and its RNA complement. This verifies that the antisense compound has been taken up by the target tissue and allows the practitioner to monitor the effectiveness of the treatment method, e.g. the effectiveness of various modes of administration, and dosages giving maximal or near-maximal levels of heteroduplex in the urine.

IX. Formulations

A morpholino antisense oligonucleotide composition may be administered in any convenient physiologically acceptable vehicle. Examples of standard pharmaceutically accepted carriers include saline, phosphate buffered saline (PBS), water, aqueous ethanol, emulsions such as oil/water emulsions, triglyceride emulsions, wetting agents, tablets and capsules. It will be understood that the choice of suitable physiologically acceptable carrier will vary dependent upon the chosen mode of administration.

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In general, in addition to the active compounds, the pharmaceutical compositions of the invention may contain suitable excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Suitable excipients include fillers such as sugars, for example, lactose, sucrose, mannitol or sorbitol, cellulose preparations, calcium phosphates, and binders such as starch, gelatin, methyl cellulose, hydroxypropyl methylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added, such as the above-mentioned starches as well as carboxymethyl starch, cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof. Auxiliaries include flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, and/or polyethylene glycol.

For oral administration, dragee cores may be provided with suitable coatings which are resistant to gastric juices. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethyl-cellulose phthalate, are used. Concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethyleneglycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of the active compounds with a suppository base. Suitable suppository bases include natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols, or higher alkanols. In addition, it is possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable liquid formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble or water-dispersible form. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, and/or dextran. The suspension may also contain stabilizers.

In addition to administration with conventional carriers, the active ingredients may be administered by a variety of specialized delivery techniques. For example, the compounds of the present invention may be administered encapsulated in liposomes. (See, e.g., Williams, S.A., *Leukemia* 10(12):1980-1989, 1996; Lappalainen et al., *Antiviral Res.* 23:119, 1994; Uhlmann et al., "Antisense Oligonucleotides: A New Therapeutic Principle", in *Chemical Reviews*, Vol. 90, No. 4, pp 544-584, 1990; Gregoriadis, G., Chapter

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14, "Liposomes", in *Drug Carriers in Biology and Medicine*, pp 287-341, Academic Press, 1979.) The active ingredient, depending upon its solubility, may be present both in the aqueous phase and in the lipidic layer(s), or in what is generally termed a liposomal suspension. The lipidic layer generally comprises phospholipids, such as lecithin or sphingomyelin, steroids such as cholesterol, ionic surfactants such as diacetylphosphate, stearylamine, or phosphatidic acid, and/or other hydrophobic materials. The diameters of the liposomes generally range from about 15 nm to about 5 microns.

Hydrogels may also be used as vehicles for antisense oligomer administration, for example, as described in WO 93/01286. Alternatively, the oligonucleotides may be administered in microspheres or microparticles. (See, e.g., Wu G Y and Wu C H, *J. Biol. Chem.* 262:4429-4432, 1987.) Such vehicles are particularly suited for topical administration or in treating restenosis.

Transdermal delivery of antisense oligomers may be accomplished by use of a pharmaceutically acceptable carrier adapted for topical administration. One example of morpholino oligomer delivery is described in PCT patent application WO 97/40854. Other sustained release compositions are also contemplated within the scope of this application.

These may include semipermeable polymeric matrices in the form of shaped articles such as films or microcapsules.

Methods for preparing such dosage forms are known or will be apparent to those skilled in the art; for example, see *Remington's Pharmaceutical Sciences* (19th Ed., Williams & Wilkins, 1995). The pharmaceutical preparations are manufactured according to procedures well known in the art. For example, they may be made by means of conventional mixing, granulating, dragee-making, dissolving, or lyophilizing processes. The process to be used will depend ultimately on the physical properties of the active ingredient used.

Materials and Methods

Oligomers

PMO's were synthesized at AVI BioPharma by methods described, for example, in Summerton and Weller, *Antisense & Nucleic Acid Drug Dev.* 7:187-95, 1993; U.S. Pat. No. 5,185,444, 1997. The oligomers were purified by ion exchange chromatography and analyzed for purity by high-performance liquid chromatography (HPLC) and mass spectrometry. The amount of full-length product was generally >90%. Before use, they were prepared as concentrated stock solutions with distilled water and stored at 4° C.

Cell Culture

Cells were obtained from the American Type Culture Collection (ATCC, Rockville, Md.) or were derived in this laboratory. They were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F-12 supplemented with glutamine (2 mM), streptomycin (100 µg/ml), and penicillin (100 U/ml). Dialyzed fetal bovine serum (FBS) was purchased from either Sigma (St. Louis, Mo.) or Hyclone (Ogden, Utah). WI-38 and HeLa cells were cultured in 10% serum and NRK cells were cultured in 4% serum.

Recombinant Plasmids and Cell Lines

The human myc genomic clone pHSR-1 was a deposit from M. Bishop to the ATCC. The 2.2-kilobase (kb) 5'-end was adapted for cloning into a luciferase vector by PCR using appropriate restriction sites incorporated into the primers (Scharf, 1990). The luciferase vector was adapted from one supplied by Clontech Inc. (Palo Alto, Calif.) to allow N-terminal fusion proteins with insect luciferase. Plasmids

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were introduced into HeLa cells using the Lipofectin protocol and reagent from Life Science Technologies (Gaithersburg, Md.) using the neomycin gene/Geneticin selection procedure (F Colbere-Garapin et al., *J Mol Biol.* 150:1-14, 1981). The rabbit α -globin-luciferase construct-containing cell line has been described (M Partridge et al., *Antisense & Nucleic Acid Drug Dev.* 6:169-75, 1996).

[³H]Thymidine incorporation Assay

Cells were trypsinized, counted, and plated at 400,000 cells/2 ml/well into 6-well dishes. On day 2, PMO antisense agents were added to the desired concentration, and the cells were scrape loaded (P L McNeil et al., *J Cell Biol.* 98:1556-64, 1984; Partridge et al., cited above) with a rubber policeman using a gentle sweeping motion. The cell suspension was pipetted once to partly disaggregate any clumps formed, and 1 ml was transferred to a 24-well dish containing an additional 1 ml/well of fresh medium. For NRK cells, on day 4, 1 μ Ci of [³H]thymidine (DuPont, NEN, Wilmington, Del.) (NET-027) was added per well, and 6 hours later, the cells were washed twice with phosphate-buffered saline (PBS), precipitated with 5% trichloroacetic acid, washed a further two times with PBS, solubilized with 0.2 N NaOH/0.1% SDS, and the amount of radioactivity was incorporated into DNA quantitated in a scintillation counter. The quantitation procedure was identical for WI-38 cells except that the incubation period for incorporation was 15 hours. In general, each agent or concentration was assayed in duplicate, and the values were averaged. The duplicates were usually within 10% of each other.

Luciferase Assay

The myc-luciferase HeLa cells were scrape loaded in a manner similar to the normal fibroblasts with the following changes. One million cells were plated into 6-well dishes, and on day 2 the entire 2 ml volume was transferred to another 6-well dish. Thirty hours later, the cells were collected and assayed for luciferase light production as described (Partridge et al.;

1996; Summerton et al., 1997; cited above).

Cell Cycle Analysis

Cells were analyzed for cell cycle stage by flow cytometry. The cells were scrape loaded as described, and two wells were combined and replated in 10-cm dishes to obtain enough cells for analysis by fluorescence-activated cell sorting (FACS). Two days later, the cells were trypsinized, washed with PBS and resuspended in cold 80% ethanol for at least 2 hours. Following the fixation step, the cells were collected by centrifugation and stained with the DNA fluorochrome propidium iodide (Telford et al., cited above). The ethanol-treated pellet was resuspended in 1 ml of 1 mM EDTA, 50 μ g/ml propidium iodide, 1 μ l/ml Triton X-100, and 10 μ g/ml RNase A. After at least 1 hour at ambient temperature, the cell suspension was analyzed using a

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Coulter Epic XL-MCL flow cytometer (Coulter Electronics, Hialeah, Fla.) with an exciting wavelength of 488 nm. Data were analyzed using Phoenix Systems (San Jose, Calif.) multicycle program software.

mRNA Reverse Transcriptase-polymerase Chain Reaction (RT-PCR) Analysis

To examine the effects of Morpholino oligomers on myc RNA splicing, HeLa cells were plated in 6-well dishes at 1 million cells/well. The next day, oligomers at 20 μ M final concentration were added to the medium and scrape loaded into the cells as detailed previously. Twenty-four hours later, the loaded cells were harvested by trypsinization and RNA prepared. Two wells were combined for each sample. Cytoplasmic (mature) RNA was extracted from the cell pellet by a Triton X-100 lysis procedure using a Qiagen Rneasy Mini Kit (Chatsworth, Calif.) following the directions for "isolation of RNA from the cytoplasm of animal cells." The RNA was eluted in 30 μ l of water with a yield of about 10-20 μ g.

Six microliters of RNA (2-3 μ g) was reverse transcribed in a final 20 μ l reaction mixture with 1X PCR buffer (10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂) (Perkin-Elmer, Norwalk, Conn.) 1 mM of each deoxynucleotide triphosphate, 0.75 μ g 9-mer random primers, and 250 U Moloney murine leukemia virus (MmuLV) RT (New England BioLabs, Beverly, Mass.). After addition of enzyme, the reactions were incubated for 10 minutes at 25° C., following by 30 minutes at 42° C., and 4 minutes to denature the polymerase at 94° C.

Exon 1-exon 2 PCR was performed using a two-step nested PCR procedure. Step 1 primers had the sequences 5'-CGG GCA CTT TGC ACT GAA ACT TAC AAC ACC (SEQ ID NO: 51) and 5'-GGT CGC AGA TGA AAC TCT GGT T (SEQ ID NO: 52). One microgram of each of the primers was added to the 20 μ l RT reaction, and the volume was adjusted to 100 μ l with 1X PCR buffer. Four units of Amplitaq (Perkin-Elmer) Taq polymerase were added, and 30 cycles were performed with steps of 94° C. for 30 seconds, 62° C. for 30 seconds, and 72° C. for 40 seconds. Step 2 used primer sequences of 5'-CTC CTT GCA GCT GCT TAG ACG CTG G (SEQ ID NO: 53) and 5'-GAA GGG TGT GAC CGC AAC GTA GGA G (SEQ ID NO: 54). The step 1 reaction mixture (4 μ l) was added to 96 μ l of 1X PCR buffer with 200 nM of each triphosphate and 1 μ g of each primer. Step 2 PCR conditions were 30 cycles of 94° C. for 30 seconds, 68° C. for 40 seconds, and 74° C. for 30 seconds. Aliquots were then analyzed by agarose gel electrophoresis.

Although the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE TABLE

SEQ ID NO	Sequence (5' → 3')	Target#	Genbank Acc. No.	Location
1	CTGTGCTTAC/CGGGTTTCCACCTCCC	Rat c-myc (SD)	Y00396	2553-79
2	ATCGTCGTGACTGT/CTGTTGGAGGG	Rat c-myc (SA)		4140-64
3	GCTCACGTTGAGGGGCATCG	Rat c-myc (ds of SA)		4161-80

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SEQUENCE TABLE				
SEQ ID NO	Sequence (5' → 3')	Target†	Genbank Acc. No.	Location
4	GGTCACTCAC/CGGTAGAGAA	Rat CYP3A2 (SD)	X62087	1155-74
5	GGGTTCCAAGT/CTATAAAGG	Rat CYP3A2 (SA)		1526-45
6*	TGTGTCTTTTCCAG	Human androgen receptor exon 2	M35845	31-44
7*	TTTGGAGACTGCCAGGGACCATG	Human androgen receptor exon 2		45-67
8	CATGGTCCCTGGCAGTCTCC	Human androgen receptor exon 2		48-67
9	TCAATGGGCAAAACATGGTCCCTGGCAGTCTCCAAA	Human androgen receptor exon 2		45-80
10*	TTTGTGTCTCTCCAG	Human androgen receptor exon 3	M35846	28-43
11*	GGAAACAGAAGTACCTGTGCGCC	Human androgen receptor exon 3		44-66
12	GGCGCACAGGTACTTCTG	Human androgen receptor exon 3		49-66
13	AATCATTTTCTGCTGGCGCACAGGTACTTCTGTTTC	Human androgen receptor exon 3		44-79
14	CCCCTGCAGCACGCGGGT	Human HCG-β subunit	X00266	1321-38
15	GAGGCAGGGCCGGCAGGACCCCTGCAGCACGCGGGT	Human HCG-β subunit		1321-57
16	GGCATCGTCGCGGGAGGCTG	Human c-myc	J00120	4506-25
17	GGGCATCGTCGCGGGAGGCT	"		4507-26
18	GGGGCATCGTCGCGGGAGGC	"		4508-27
19	AGGGGCATCGTCGCGGGAGG	"		4509-28
20	GAGGGGCATCGTCGCGGGAG	"		4510-29
21	TGAGGGGCATCGTCGCGGGA	"		4511-30
22	TTGAGGGGCATCGTCGCGGG	"		4512-31
23	GTTGAGGGGCATCGTCGCGG	"		4513-32
24	CGTTGAGGGGCATCGTCGCG	"		4514-33
25	ACGTTGAGGGGCATCGTCGC	"		4515-34
26	AACGTTGAGGGGCATCGTCG	"		4516-35
27	TAACGTTGAGGGGCATCGTC	"		4517-36
28	CTAACGTTGAGGGGCATCGT	"		4518-37
29	GCTAACGTTGAGGGGCATCG	"		4519-38
30	AGCTAACGTTGAGGGGCATC	"		4520-39
31	AAGCTAACGTTGAGGGGCAT	"		4521-40
32	GAAGCTAACGTTGAGGGGCA	"		4522-41
33	TCCTCATCTTCTTGTTCTC	"		6656-75
34	AACAACATCGATTCTTCTCCTCATCTTCTTGTTCTC	"		6656-91
35	CCCGGAAGGCAGTCTGGC	Human p53	X54156	11691-708

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SEQUENCE TABLE				
SEQ ID NO	Sequence (5' → 3')	Target†	Genbank Acc. No.	Location
36	TCCTCCATGGCAGTGACCCGGAAGGCAGTCTGGCTG	"		11689-724
37	CTACTGGCCGCTGAAGGGC	Human abl (ds of bcr-abl fusion point)	AJ131466	376-94
38	GCTCAAAGTCAGATGCTACTGGCCGCTGAAGGGCTT	Human abl (ds of bcr-abl fusion point)		374-409
39	TCGTCGGTCTCTCCGCTTCTTCTTGCC	HIV-1 rev (prior art)	U69590	5517-43
40	CTCTGGTGGTGGTAAGGGT	HIV-1 rev	L39106	7885-7904
41	CGGGTCTGTCGGGTTCCCTCTGGTGGTGGTAAGGGT	"		7885-7921
42	GGGGCAUCGUCGUGACUGU/CUGUUGGAGGG	Rat c-myc (SA)	Y00396	4140-69
43	CGUCGUGACUGU/CUGUUGGAGG	"	Y00396	4141-62
44	CGTCGTGACTGT/CTGTTGGAGG	"	Y00396	4141-62
45	GGCAUCGUCGCGGGAGGCUG/CUGGAGCG	Human c-myc (SA)	J00120	4498-4505
46	CCGCGACAUAGGACGGAGAGCAGAGCCC	Rat c-myc	Y00396	4364-91
47	ACTGTGAGGGCGATCGCTGC (scrambled)	derived from SEQ ID NO: 25		
48	ACGATGACTGGCATAGTCGC (3 mismatches)	derived from SEQ ID NO: 25		
49	CTCCGCAATGCTGAAAGGTG	Rat BCL-2 (cntrl)		
50	GGCGUGCCUCAACAUGGUGGCGG	Rat PCNA-1 (cntrl)		
51	CGGGCACTTTGCACTGAAACTTACAACACC	primer sequence		
52	GGTCGCAGATGAAACTCTGGTT	"		
53	CTCCTTGCAGCTGCTTAGACGCTGG	"		
54	GAAGGGTGTGACCGCAACGTAGGAG	"		

*native sequence, not antisense

†unless otherwise indicated, antisense target is downstream (ds) of splice
acceptor (SA) junction;

SD = splice donor junction

SEQUENCE LISTING

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<220> FEATURE:

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<223> OTHER INFORMATION: antisense

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<210> SEQ ID NO 11
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<400> SEQUENCE: 11

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<400> SEQUENCE: 15

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gaggggcac gtcgcggag 20

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tgaggggcat cgtcgggga 20

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<223> OTHER INFORMATION: antisense

<400> SEQUENCE: 22

ttgaggggca tcgtcgcggg 20

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: antisense

<400> SEQUENCE: 23

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<220> FEATURE:

<223> OTHER INFORMATION: antisense

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<212> TYPE: DNA

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<220> FEATURE:

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acgttgaggg gcatcgtcgc 20

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<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: antisense

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aacgttgagg ggcacgtcgc 20

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<220> FEATURE:

<223> OTHER INFORMATION: antisense

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<220> FEATURE:

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ctaacgttga gggcacgtcgc 20

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<220> FEATURE:
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<223> OTHER INFORMATION: antisense

<400> SEQUENCE: 31

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<220> FEATURE:
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<400> SEQUENCE: 32

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<220> FEATURE:
<223> OTHER INFORMATION: antisense

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<400> SEQUENCE: 35

cccgggaaggc agtctggc 18

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 <223> OTHER INFORMATION: antisense

<400> SEQUENCE: 37

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<210> SEQ ID NO 38
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: antisense

<400> SEQUENCE: 38

gctcaaagtc agatgctact ggcgcgtgaa gggctt 36

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tcgtcgttct ctccgttct tcttgcc 27

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 <212> TYPE: DNA
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 <220> FEATURE:
 <223> OTHER INFORMATION: antisense

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 <212> TYPE: DNA
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 <220> FEATURE:
 <223> OTHER INFORMATION: antisense

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<210> SEQ ID NO 42
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<212> TYPE: DNA
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<223> OTHER INFORMATION: scrambled version of SEQ ID NO: 25

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<223> OTHER INFORMATION: SEQ ID NO: 25 with three mismatched nucleotides

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<400> SEQUENCE: 50

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<220> FEATURE:
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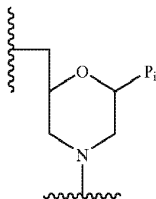
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It is claimed:

1. An antisense compound having an uncharged morpholino backbone and the base sequence presented as SEQ ID NO: 35.

2. The compound of claim 1, wherein said backbone comprises morpholino subunits, as shown in the structure below,



where P_i is a purine or pyrimidine base-pairing moiety effective to bind to a base in a polynucleotide, and

uncharged phosphorus-containing linkages, one to three atoms long, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit.

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3. The compound of claim 2, wherein the each said phosphorus-containing linkage is a phosphorodiamidate linkage as represented by —P(=Z)(X)—Y— , where X=NH_2 , NHR , or NRR' , Y=O , and Z=O , or where X=1 OR , Y=NH or NR' , and Z=O , and R and R' are groups which do not interfere with target binding.

4. The compound of claim 3, wherein R and R' are moieties independently selected from alkyl, polyalkyleneoxy, and a combination thereof, which may be substituted with one or more groups selected from hydroxy, alkoxy, amino, alkylamino, thiol, alkanethiol, halogen, oxo, carboxylic acid, carboxylic ester, and inorganic ester.

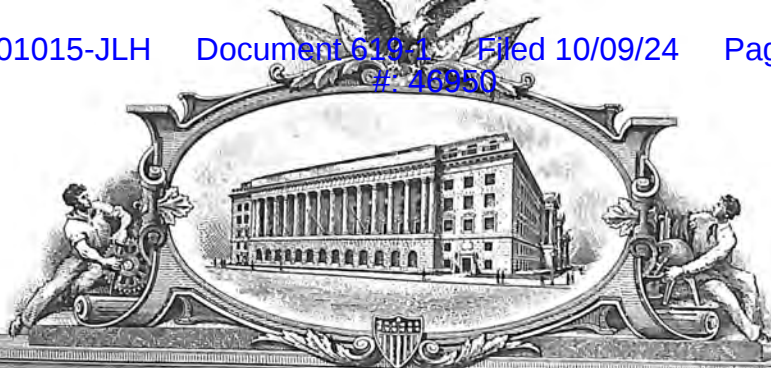
5. The compound of claim 4, wherein each said moiety R and R' , independent of substitution, is from 1 to 6 atoms long.

6. The compound of claim 3, wherein NRR' represents a nitrogen heterocycle having 5–7 ring atoms selected from nitrogen, carbon, oxygen, and sulfur, and having at least as many carbon ring atoms as non-carbon ring atoms.

* * * * *

Exhibit 9

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TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

April 13, 2022

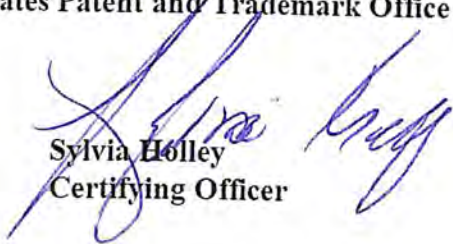
THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THIS OFFICE OF:

PATENT NUMBER: 9,994,851

ISSUE DATE: June 12, 2018

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office




Sylvia Holley
Certifying Officer

46851

US009994851B2

(12) **United States Patent**
Wilton et al.(10) **Patent No.:** **US 9,994,851 B2**
(45) **Date of Patent:** ***Jun. 12, 2018**(54) **ANTISENSE OLIGONUCLEOTIDES FOR
INDUCING EXON SKIPPING AND
METHODS OF USE THEREOF**

(56)

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Crawley (AU)(72) Inventors: **Stephen Donald Wilton, Applecross**
(AU); Sue Fletcher, Bayswater (AU);
Graham McClorey, Bayswater (AU)(73) Assignee: **The University of Western Australia,**
Crawley (AU)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days. days.This patent is subject to a terminal dis-
claimer.(21) Appl. No.: **15/705,172**(22) Filed: **Sep. 14, 2017**(65) **Prior Publication Data**

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Sep. 23, 2016, which is a continuation of application
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9,605,262, which is a continuation of application No.
13/741,150, filed on Jan. 14, 2013, now abandoned,
which is a continuation of application No.
13/168,857, filed on Jun. 24, 2011, now abandoned,
which is a continuation of application No.
12/837,359, filed on Jul. 15, 2010, now Pat. No.
8,232,384, which is a continuation of application No.
11/570,691, filed as application No.
PCT/AU2005/000943 on Jun. 28, 2005, now Pat. No.
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C07H 21/04 (2006.01)
C12N 15/113 (2010.01)(52) **U.S. Cl.**
CPC **C12N 15/113** (2013.01); **C12N 2310/11**
(2013.01); **C12N 2310/315** (2013.01); **C12N**
2310/321 (2013.01); **C12N 2310/323**
(2013.01); **C12N 2310/33** (2013.01); **C12N**
2310/3341 (2013.01); **C12N 2310/3519**
(2013.01); **C12N 2320/30** (2013.01); **C12N**
2320/33 (2013.01)(58) **Field of Classification Search**
CPC **C07H 21/04**
See application file for complete search history.

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Primary Examiner — Kimberly Chong

(74) Attorney, Agent, or Firm — Sterne, Kessler,
Goldstein & Fox P.L.L.C.(57) **ABSTRACT**An antisense molecule capable of binding to a selected target
site to induce exon skipping in the dystrophin gene, as set
forth in SEQ ID NO: 1 to 214.**2 Claims, 22 Drawing Sheets**

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FIGURE 1

bp Acceptor ESE Donor

ucaugcacugagugacccucuuucucgcagGCGCUAGCUGGAGCA////CCGUGCAGACUGACGGucucau

SEQ ID NO:214

SEQ ID NO:213

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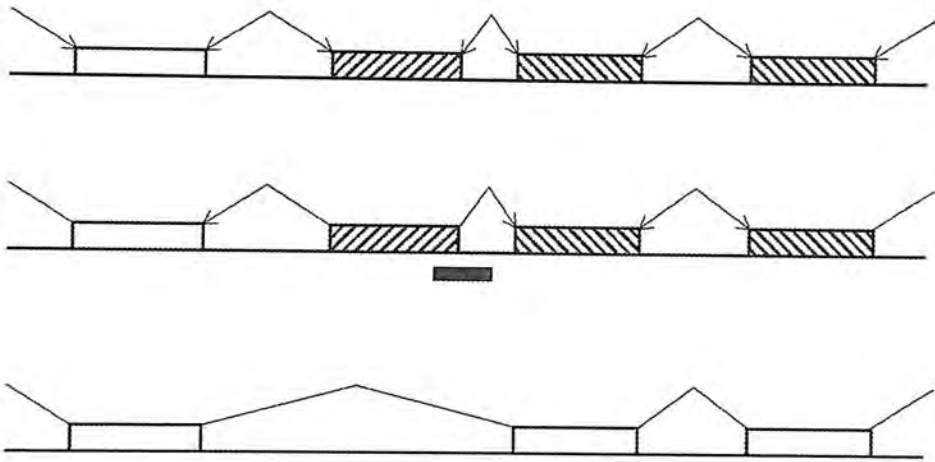


FIGURE 2

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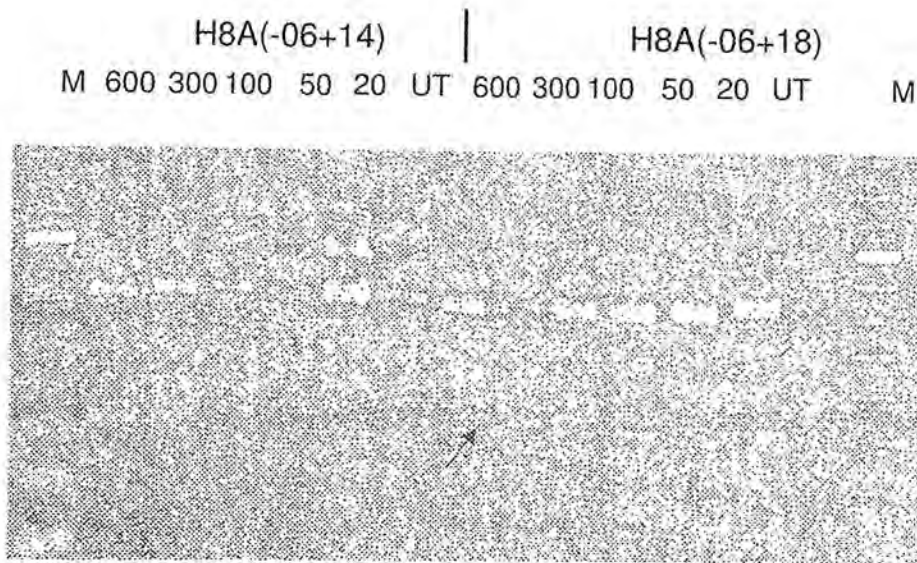


FIGURE 3

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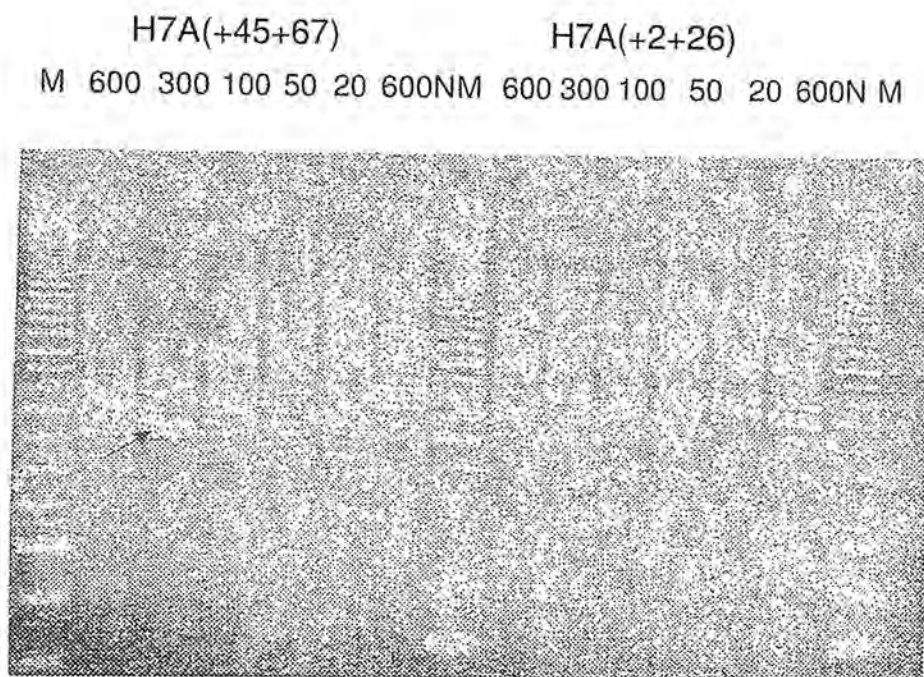


FIGURE 4

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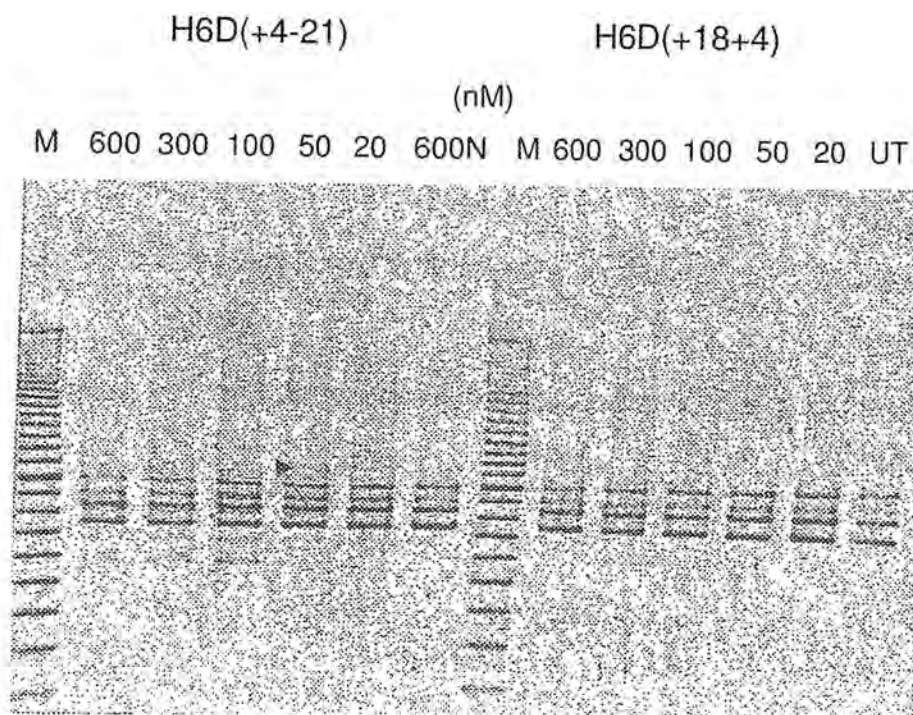


FIGURE 5

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6A(+69+91)

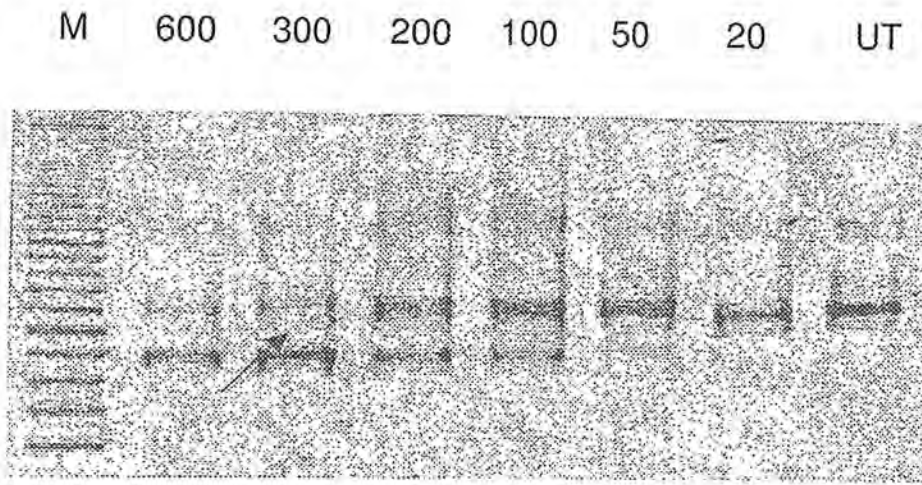


FIGURE 6

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H4A(+13+32)

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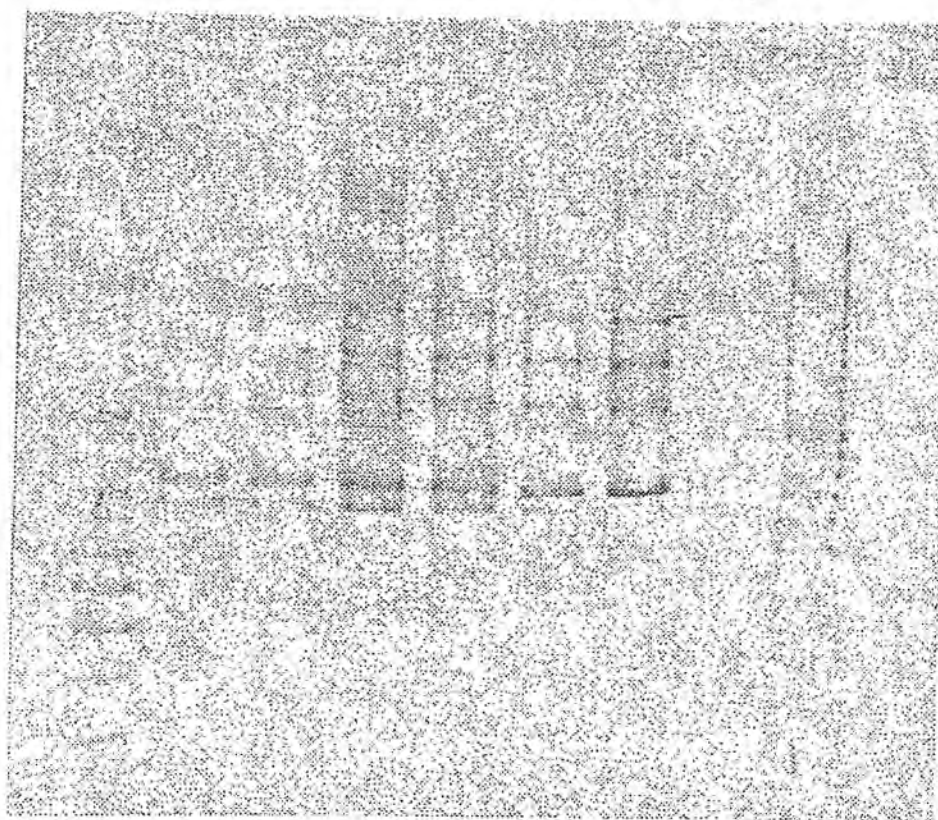


FIGURE 7

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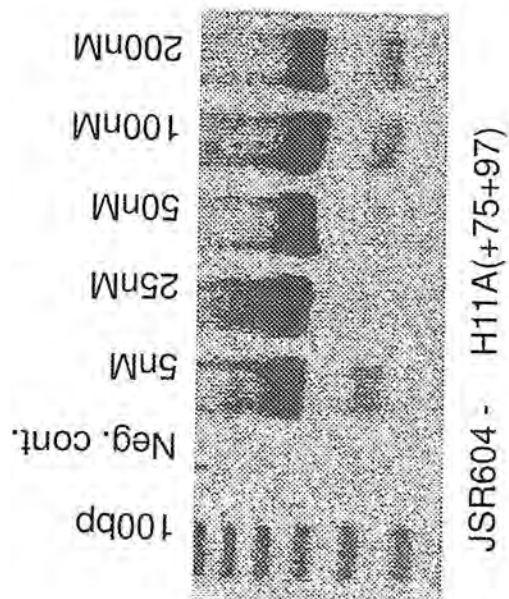


FIGURE 8B

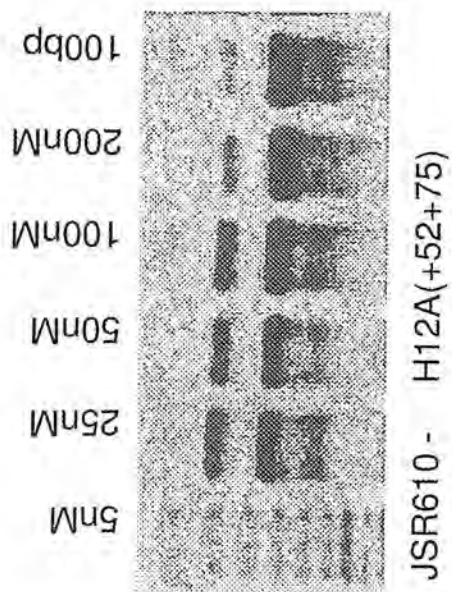


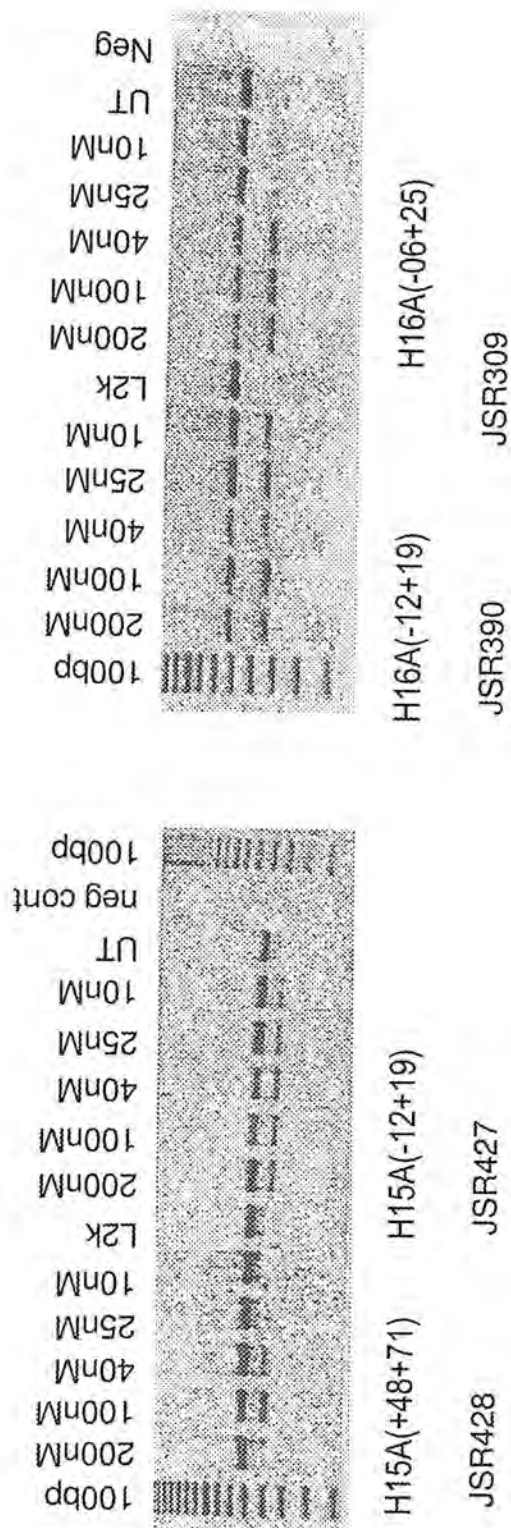
FIGURE 8A

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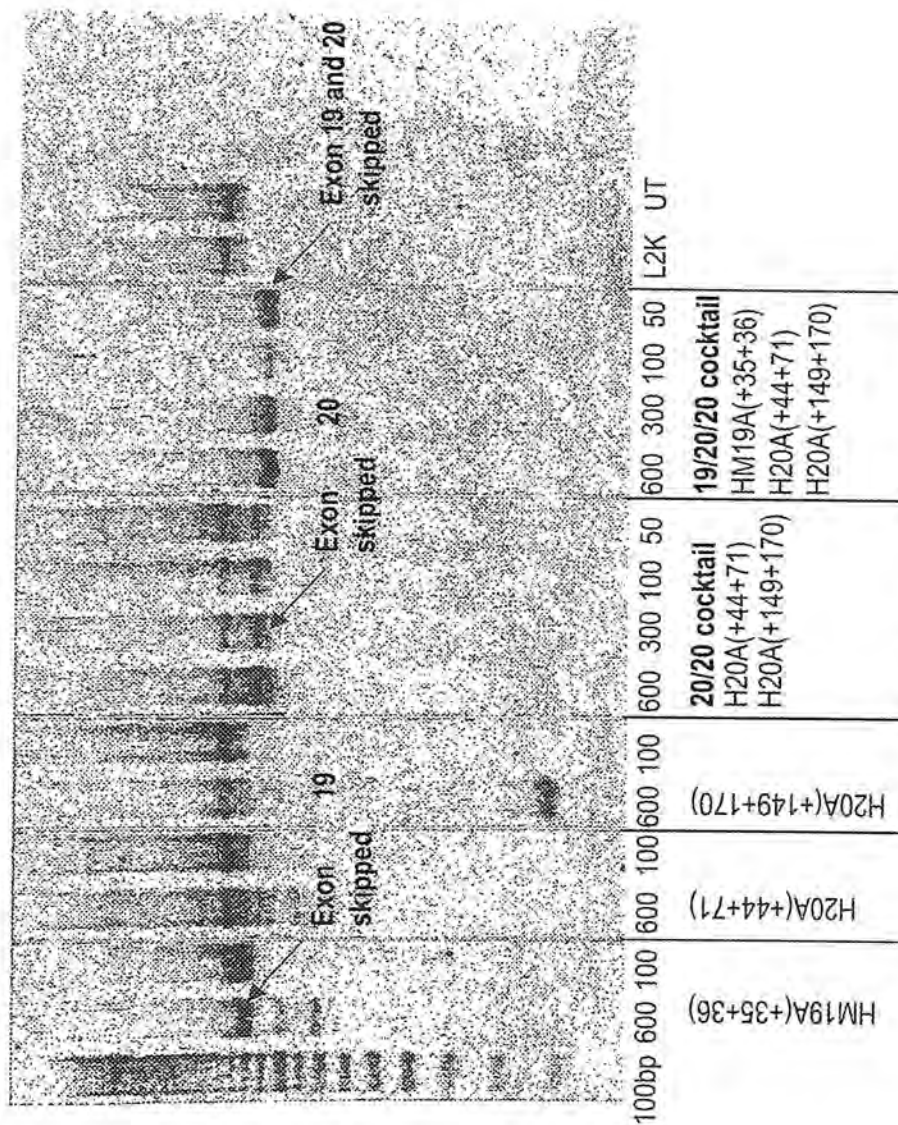


FIGURE 10

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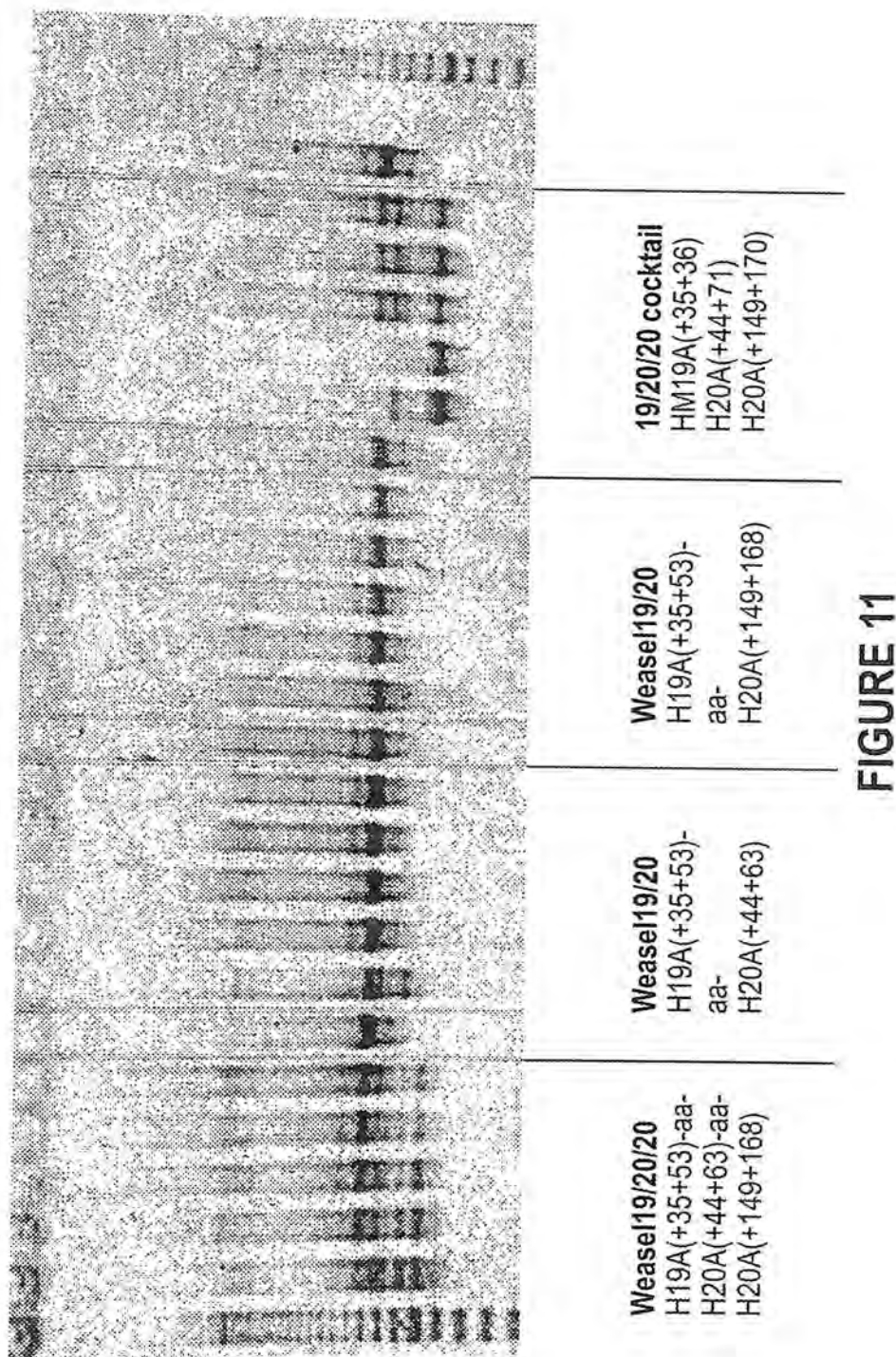


FIGURE 11

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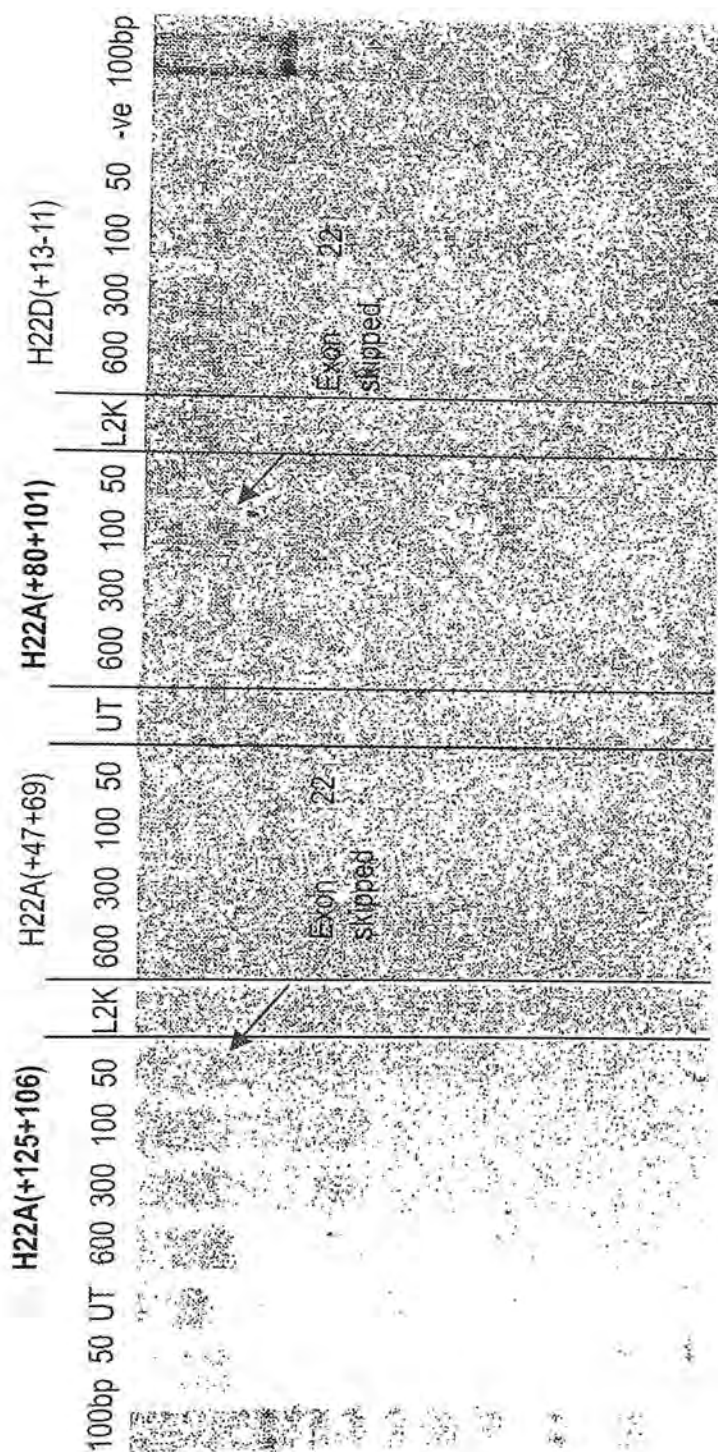


FIGURE 12

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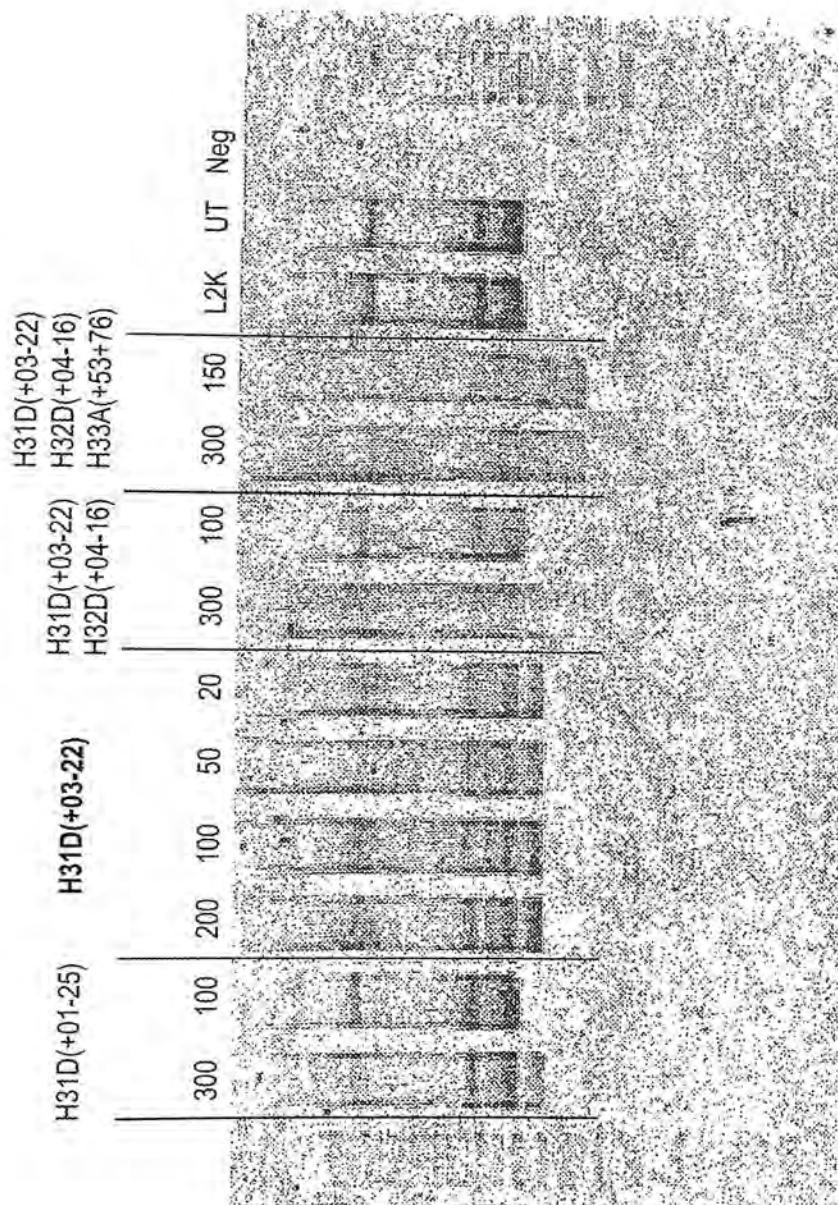


FIGURE 13

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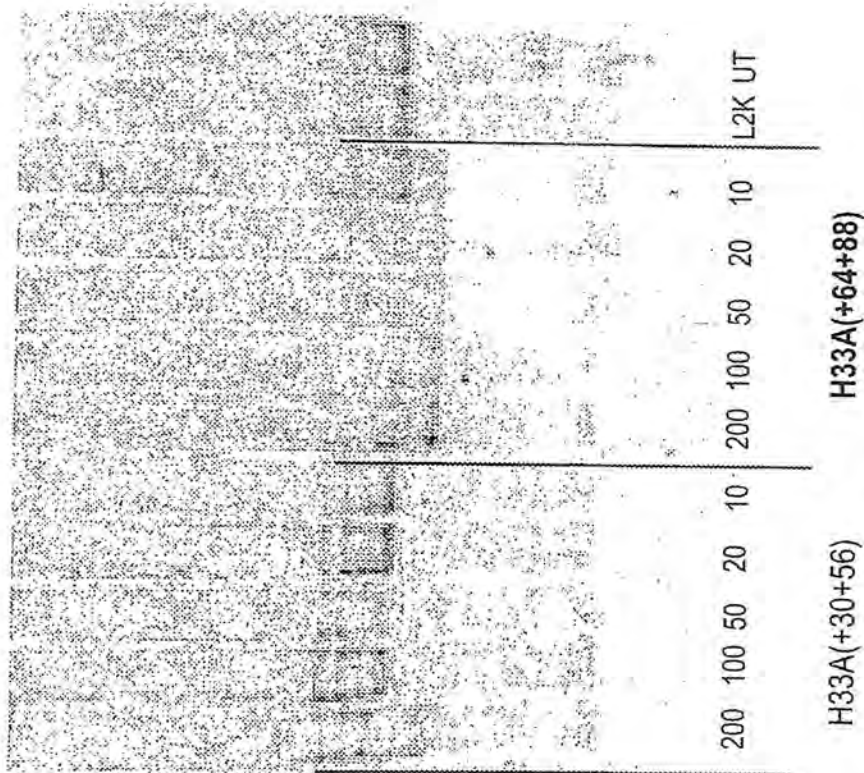


FIGURE 14

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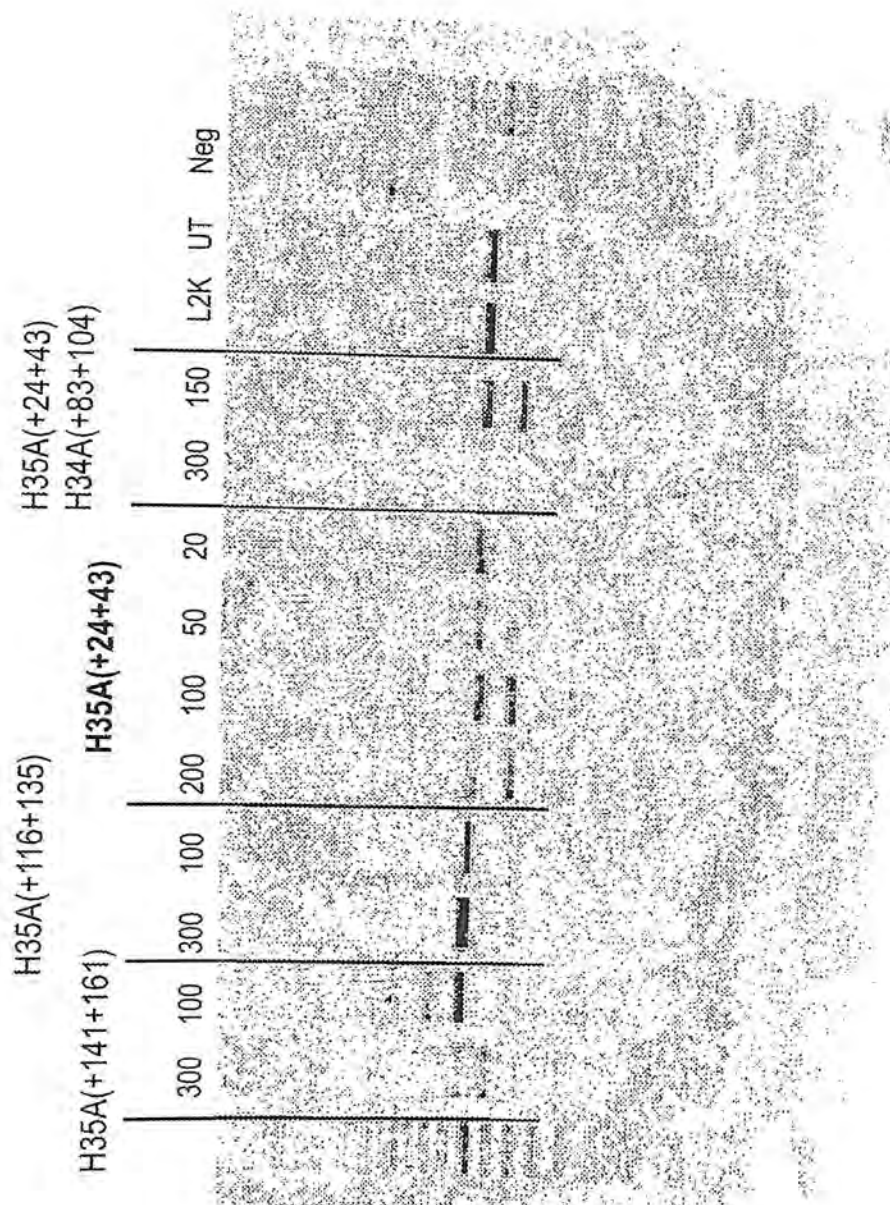


FIGURE 15

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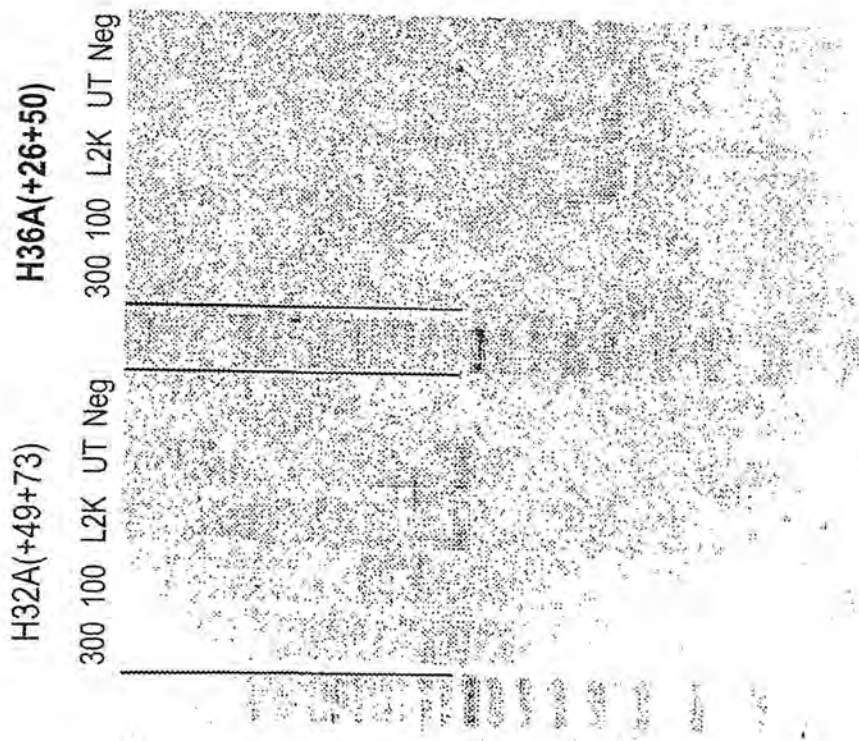


FIGURE 16

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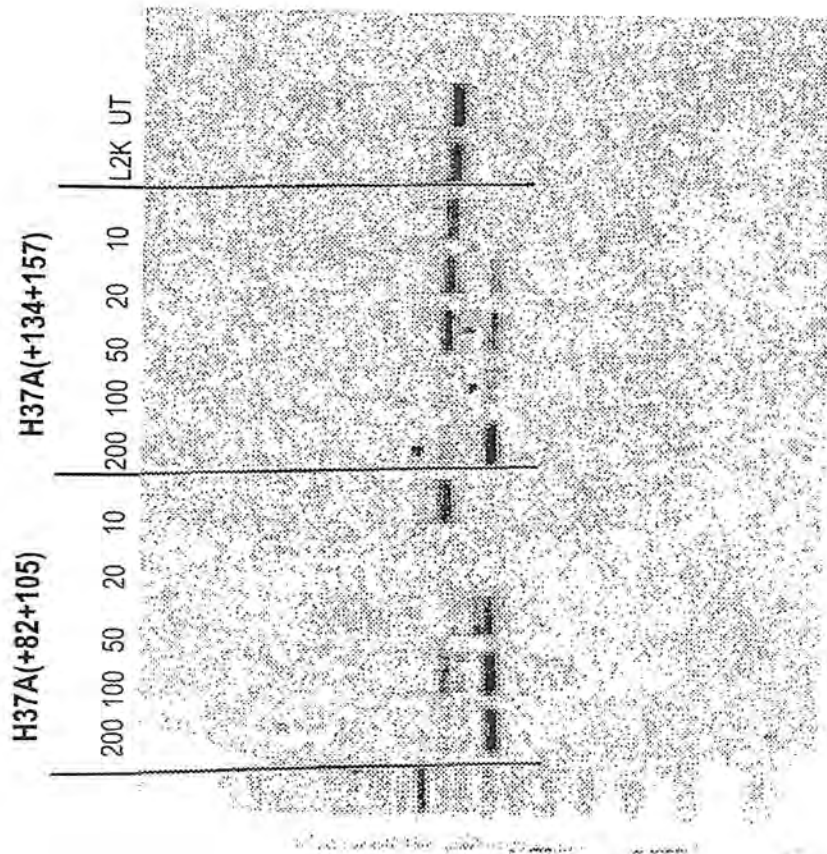


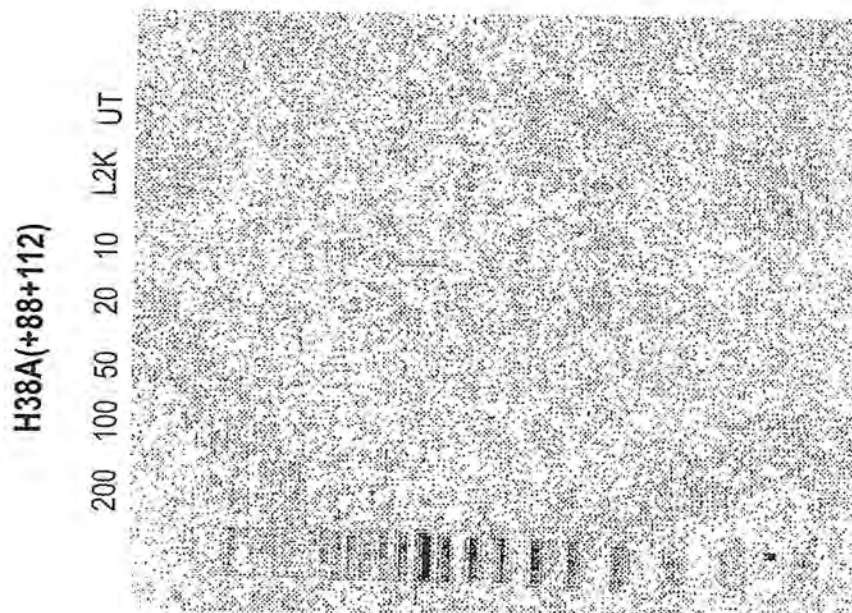
FIGURE 17

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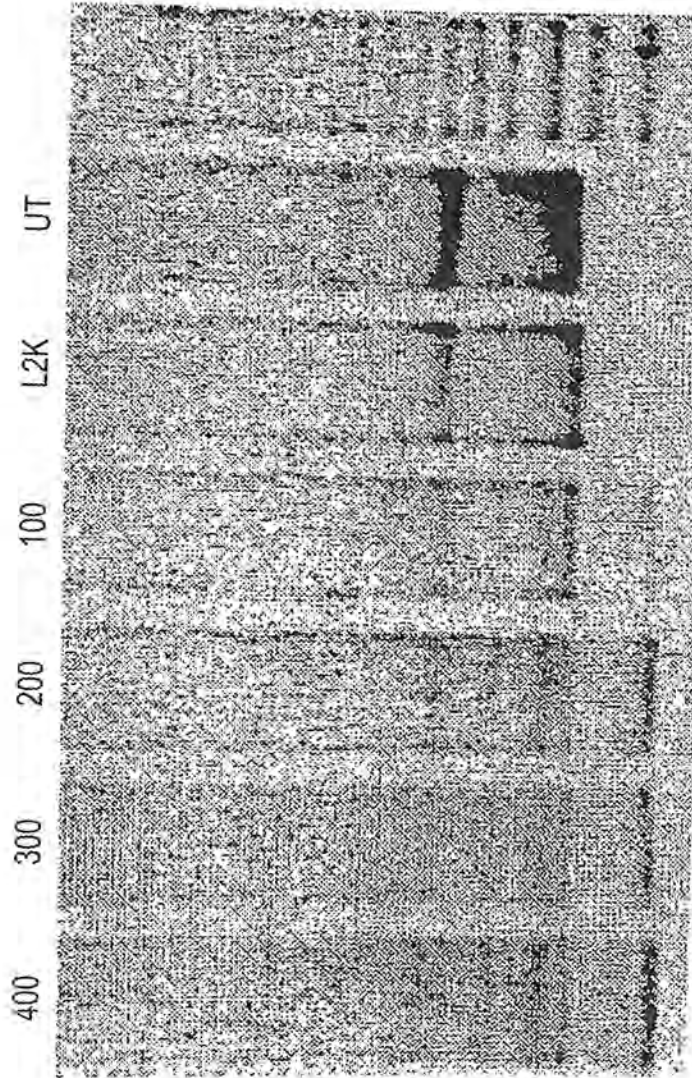


FIGURE 19

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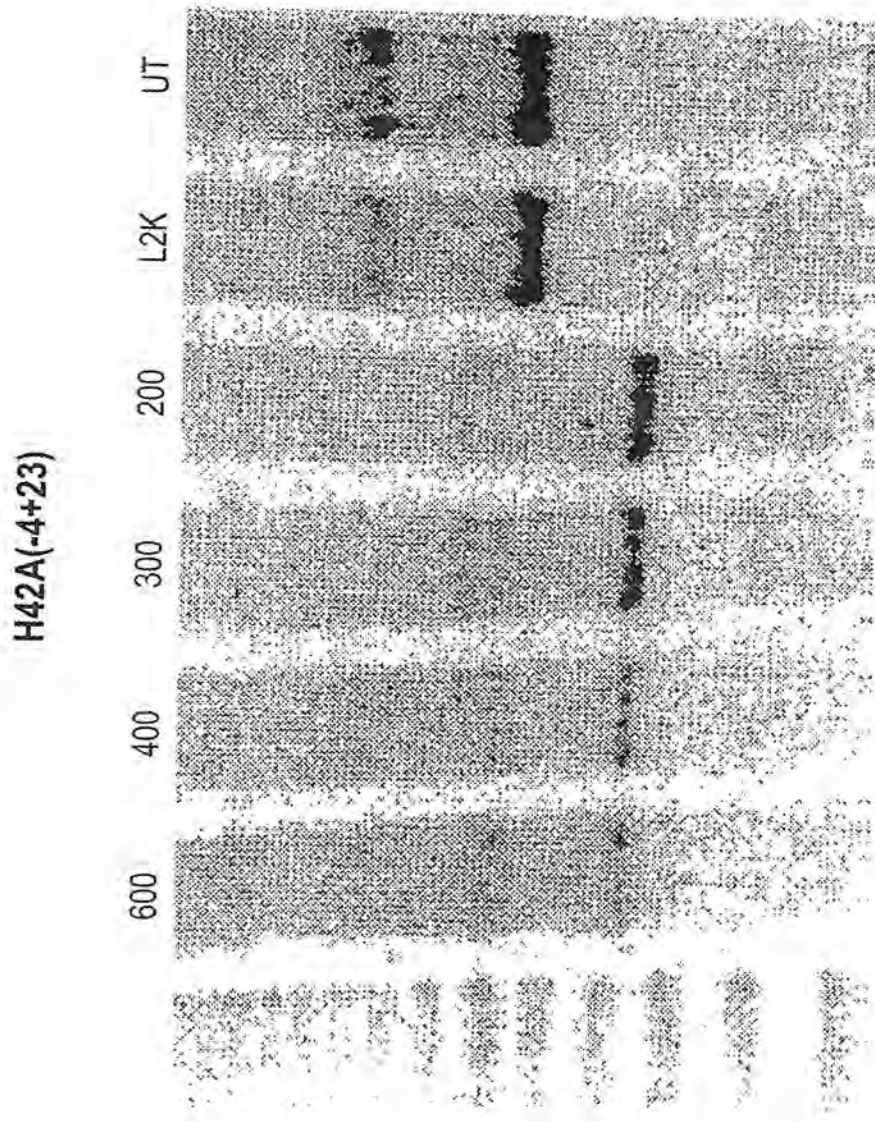


FIGURE 20

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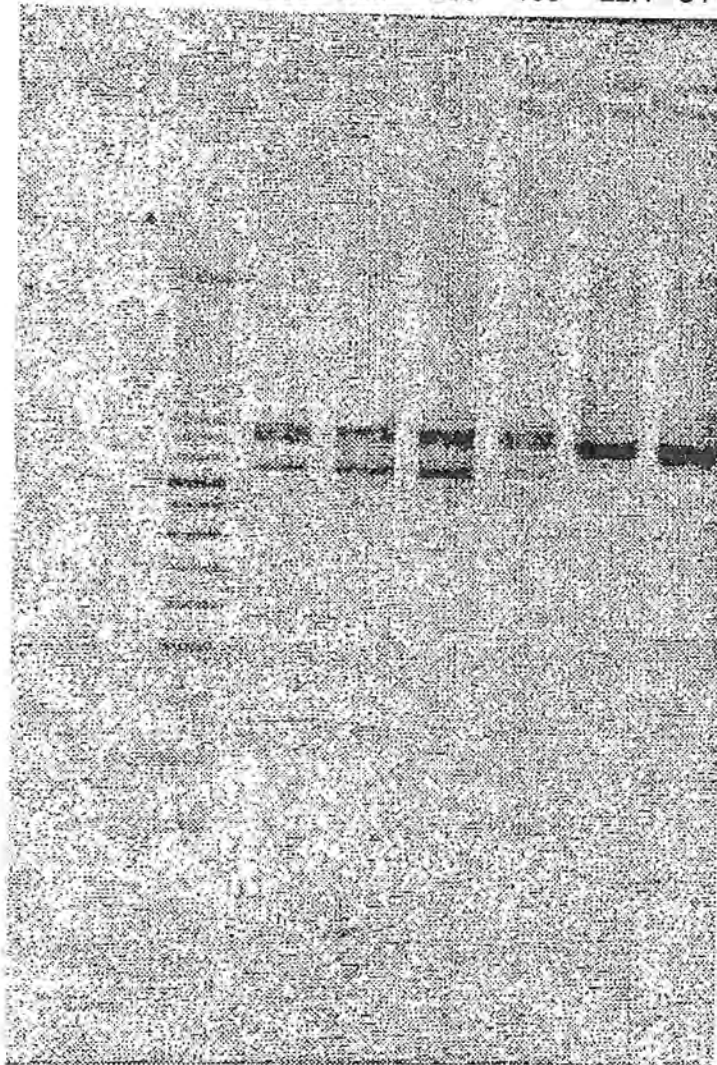


FIGURE 21

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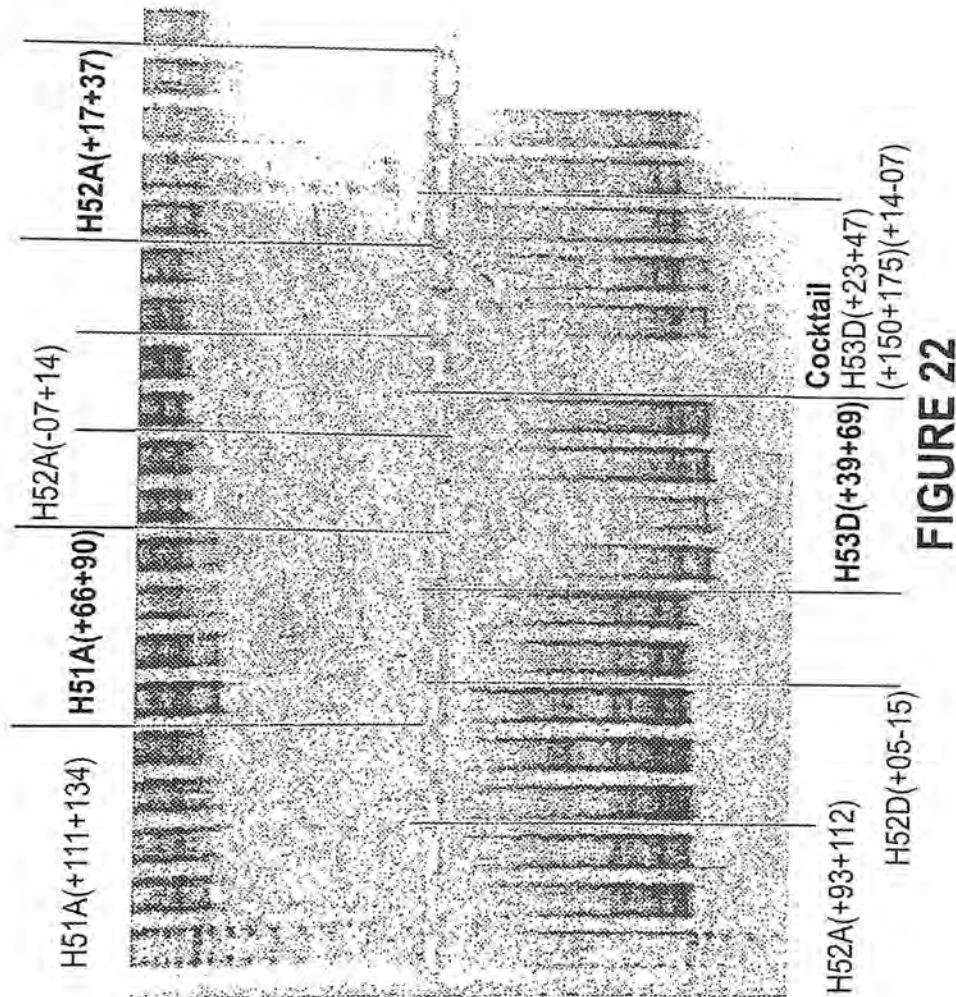


FIGURE 22

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1

ANTISENSE OLIGONUCLEOTIDES FOR INDUCING EXON SKIPPING AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 15/274,772, filed Sep. 23, 2016, now pending, which application is a continuation of U.S. patent application Ser. No. 14/740,097, filed Jun. 15, 2015, now issued as U.S. Pat. No. 9,605,262, which application is a continuation of U.S. patent application Ser. No. 13/741,150, filed Jan. 14, 2013, now abandoned, which application is a continuation of U.S. patent application Ser. No. 13/168,857, filed Jun. 24, 2011, now abandoned, which application is a continuation of U.S. patent application Ser. No. 12/837,359, filed Jul. 15, 2010, now issued as U.S. Pat. No. 8,232,384, which application is a continuation of U.S. patent application Ser. No. 11/570,691, filed Jan. 15, 2008, now issued as U.S. Pat. No. 7,807,816, which application is a 35 U.S.C. § 371 National Phase Application of PCT/AU2005/000943, filed Jun. 28, 2005, which claims priority to Australian Patent Application No. 2004903474, filed Jun. 28, 2004; which applications are each incorporated herein by reference in their entireties.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with the application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is AVN-008CN41_Sequence-Listing.txt. The text file is 62,086 Kilobytes, was created on Sep. 14, 2017 and is being submitted electronically via EFS-Web.

FIELD OF THE INVENTION

The present invention relates to novel antisense compounds and compositions suitable for facilitating exon skipping. It also provides methods for inducing exon skipping using the novel antisense compounds as well as therapeutic compositions adapted for use in the methods of the invention.

BACKGROUND ART

Significant effort is currently being expended researching methods for suppressing or compensating for disease-causing mutations in genes. Antisense technologies are being developed using a range of chemistries to affect gene expression at a variety of different levels (transcription, splicing, stability, translation). Much of that research has focused on the use of antisense compounds to correct or compensate for abnormal or disease-associated genes in a myriad of different conditions.

Antisense molecules are able to inhibit gene expression with exquisite specificity and because of this many research efforts concerning oligonucleotides as modulators of gene expression have focused on inhibiting the expression of targeted genes such as oncogenes or viral genes. The antisense oligonucleotides are directed either against RNA (sense strand) or against DNA where they form triplex structures inhibiting transcription by RNA polymerase II. To achieve a desired effect in specific gene down-regulation, the oligonucleotides must either promote the decay of the tar-

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geted mRNA or block translation of that mRNA, thereby effectively preventing de novo synthesis of the undesirable target protein.

Such techniques are not useful where the object is to up-regulate production of the native protein or compensate for mutations which induce premature termination of translation such as nonsense or frame-shifting mutations. Furthermore, in cases where a normally functional protein is prematurely terminated because of mutations therein, a means for restoring some functional protein production through antisense technology has been shown to be possible through intervention during the splicing processes (Sierakowska H, et al., (1996) *Proc Natl Acad Sci USA* 93, 12840-12844; Wilton S D, et al., (1999) *Neuromusc Disorders* 9, 330-338; van Deutekom J C et al., (2001) *Human Mol Genet* 10, 1547-1554). In these cases, the defective gene transcript should not be subjected to targeted degradation so the antisense oligonucleotide chemistry should not promote target mRNA decay.

In a variety of genetic diseases, the effects of mutations on the eventual expression of a gene can be modulated through a process of targeted exon skipping during the splicing process. The splicing process is directed by complex multi-particle machinery that brings adjacent exon-intron junctions in pre-mRNA into close proximity and performs cleavage of phosphodiester bonds at the ends of the introns with their subsequent reformation between exons that are to be spliced together. This complex and highly precise process is mediated by sequence motifs in the pre-mRNA that are relatively short semi-conserved RNA segments to which bind the various nuclear splicing factors that are then involved in the splicing reactions. By changing the way the splicing machinery reads or recognises the motifs involved in pre-mRNA processing, it is possible to create differentially spliced mRNA molecules. It has now been recognised that the majority of human genes are alternatively spliced during normal gene expression, although the mechanisms invoked have not been identified. Using antisense oligonucleotides, it has been shown that errors and deficiencies in a coded mRNA could be bypassed or removed from the mature gene transcripts.

In nature, the extent of genetic deletion or exon skipping in the splicing process is not fully understood, although many instances have been documented to occur, generally at very low levels (Sherrat T G, et al., (1993) *Am J Hum Genet* 53, 1007-1015). However, it is recognised that if exons associated with disease-causing mutations can be specifically deleted from some genes, a shortened protein product can sometimes be produced that has similar biological properties of the native protein or has sufficient biological activity to ameliorate the disease caused by mutations associated with the target exon (Lu Q L, et al., (2003) *Nature Medicine* 9, 1009-1014; Aartsma-Rus A et al., (2004) *Am J Hum Genet* 74: 83-92).

This process of targeted exon skipping is likely to be particularly useful in long genes where there are many exons and introns, where there is redundancy in the genetic constitution of the exons or where a protein is able to function without one or more particular exons (e.g. with the dystrophin gene, which consists of 79 exons; or possibly some collagen genes which encode for repeated blocks of sequence or the huge nebulin or titin genes which are comprised of ~80 and over 370 exons, respectively).

Efforts to redirect gene processing for the treatment of genetic diseases associated with truncations caused by mutations in various genes have focused on the use of antisense oligonucleotides that either: (1) fully or partially overlap

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with the elements involved in the splicing process; or (2) bind to the pre-mRNA at a position sufficiently close to the element to disrupt the binding and function of the splicing factors that would normally mediate a particular splicing reaction which occurs at that element (e.g., binds to the pre-mRNA at a position within 3, 6, or 9 nucleotides of the element to be blocked).

For example, modulation of mutant dystrophin pre-mRNA splicing with antisense oligoribonucleotides has been reported both in vitro and in vivo. In one type of dystrophin mutation reported in Japan, a 52-base pair deletion mutation causes exon 19 to be removed with the flanking introns during the splicing process (Matsuo et al., (1991) *J Clin Invest.*, 87:2127-2131). An in vitro minigene splicing system has been used to show that a 31-mer 2'-O-methyl oligoribonucleotide complementary to the 5' half of the deleted sequence in dystrophin Kobe exon 19 inhibited splicing of wild-type pre-mRNA (Takeshima et al. (1995), *J. Clin. Invest.*, 95, 515-520). The same oligonucleotide was used to induce exon skipping from the native dystrophin gene transcript in human cultured lymphoblastoid cells.

Dunckley et al., (1997) *Nucleosides & Nucleotides*, 16, 1665-1668 described in vitro constructs for analysis of splicing around exon 23 of mutated dystrophin in the mdx mouse mutant, a model for muscular dystrophy. Plans to analyse these constructs in vitro using 2' modified oligonucleotides targeted to splice sites within and adjacent to mouse dystrophin exon 23 were discussed, though no target sites or sequences were given.

2'-O-methyl oligoribonucleotides were subsequently reported to correct dystrophin deficiency in myoblasts from the mdx mouse from this group. An antisense oligonucleotide targeted to the 3' splice site of murine dystrophin intron 22 was reported to cause skipping of the mutant exon as well as several flanking exons and created a novel in-frame dystrophin transcript with a novel internal deletion. This mutated dystrophin was expressed in 1-2% of antisense treated mdx myotubes. Use of other oligonucleotide modifications such as 2'-O-methoxyethyl phosphodiester are described (Dunckley et al. (1998) *Human Mol. Genetics*, 5, 1083-90).

Thus, antisense molecules may provide a tool in the treatment of genetic disorders such as Duchenne Muscular Dystrophy (DMD). However, attempts to induce exon skipping using antisense molecules have had mixed success. Studies on dystrophin exon 19, where successful skipping of that exon from the dystrophin pre-mRNA was achieved using a variety of antisense molecules directed at the flanking splice sites or motifs within the exon involved in exon definition as described by Errington et al. (2003) *J Gen Med* 5, 518-527".

In contrast to the apparent ease of exon 19 skipping, the first report of exon 23 skipping in the mdx mouse by Dunckley et al., (1998) is now considered to be reporting only a naturally occurring revertant transcript or artefact rather than any true antisense activity. In addition to not consistently generating transcripts missing exon 23, Dunckley et al., (1998) did not show any time course of induced exon skipping, or even titration of antisense oligonucleotides, to demonstrate dose dependent effects where the levels of exon skipping corresponded with increasing or decreasing amounts of antisense oligonucleotide. Furthermore, this work could not be replicated by other researchers.

The first example of specific and reproducible exon skipping in the mdx mouse model was reported by Wilton et al., (1999) *Neuromuscular Disorders* 9, 330-338. By directing an antisense molecule to the donor splice site, consistent and efficient exon 23 skipping was induced in the dystrophin mRNA within 6 hours of treatment of the cultured cells. Wilton et al., (1999), also describe targeting the acceptor

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region of the mouse dystrophin pre-mRNA with longer antisense oligonucleotides and being unable to repeat the published results of Dunckley et al., (1998). No exon skipping, either 23 alone or multiple removal of several flanking exons, could be reproducibly detected using a selection of antisense oligonucleotides directed at the acceptor splice site of intron 22.

While the first antisense oligonucleotide directed at the intron 23 donor splice site induced consistent exon skipping in primary cultured myoblasts, this compound was found to be much less efficient in immortalized cell cultures expressing higher levels of dystrophin. However, with refined targeting and antisense oligonucleotide design, the efficiency of specific exon removal was increased by almost an order of magnitude (see Mann C J et al., (2002) *J Gen Med* 4, 644-654).

Thus, there remains a need to provide antisense oligonucleotides capable of binding to and modifying the splicing of a target nucleotide sequence. Simply directing the antisense oligonucleotides to motifs presumed to be crucial for splicing is no guarantee of the efficacy of that compound in a therapeutic setting.

SUMMARY OF THE INVENTION

The present invention provides antisense molecule compounds and compositions suitable for binding to RNA motifs involved in the splicing of pre-mRNA that are able to induce specific and efficient exon skipping and a method for their use thereof.

The choice of target selection plays a crucial role in the efficiency of exon skipping and hence its subsequent application of a potential therapy. Simply designing antisense molecules to target regions of pre-mRNA presumed to be involved in splicing is no guarantee of inducing efficient and specific exon skipping. The most obvious or readily defined targets for splicing intervention are the donor and acceptor splice sites although there are less defined or conserved motifs including exonic splicing enhancers, silencing elements and branch points.

The acceptor and donor splice sites have consensus sequences of about 16 and 8 bases respectively (see FIG. 1 for schematic representation of motifs and domains involved in exon recognition, intron removal and the splicing process).

According to a first aspect, the invention provides antisense molecules capable of binding to a selected target to induce exon skipping.

For example, to induce exon skipping in exons 3 to 8, 10 to 16, 19 to 40, 42 to 44, 46, 47, and 50 to 53 in the Dystrophin gene transcript the antisense molecules are preferably selected from the group listed in Table 1A.

In a further example, it is possible to combine two or more antisense oligonucleotides of the present invention together to induce multiple exon skipping in exons 19-20, and 53. This is a similar concept to targeting of a single exon. A combination or "cocktail" of antisense oligonucleotides are directed at adjacent exons to induce efficient exon skipping.

In another example, to induce exon skipping in exons 19-20, 31, 34 and 53 it is possible to improve exon skipping of a single exon by joining together two or more antisense oligonucleotide molecules. This concept is termed by the inventor as a "weasel", an example of a cunningly designed antisense oligonucleotide. A similar concept has been described in Aartsma-Rus A et al., (2004) *Am J Hum Genet* 74: 83-92).

According to a second aspect, the present invention provides antisense molecules selected and or adapted to aid in the prophylactic or therapeutic treatment of a genetic disorder comprising at least an antisense molecule in a form suitable for delivery to a patient.

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According to a third aspect, the invention provides a method for treating a patient suffering from a genetic disease wherein there is a mutation in a gene encoding a particular protein and the effect of the mutation can be abrogated by exon skipping, comprising the steps of: (a) selecting an antisense molecule in accordance with the methods described herein; and (b) administering the molecule to a patient in need of such treatment.

The invention also addresses the use of purified and isolated antisense oligonucleotides of the invention, for the manufacture of a medicament for treatment of a genetic disease.

The invention further provides a method of treating a condition characterised by Duchenne muscular dystrophy, which method comprises administering to a patient in need of treatment an effective amount of an appropriately designed antisense oligonucleotide of the invention, relevant to the particular genetic lesion in that patient. Further, the invention provides a method for prophylactically treating a patient to prevent or at least minimise Duchenne muscular dystrophy, comprising the step of: administering to the patient an effective amount of an antisense oligonucleotide or a pharmaceutical composition comprising one or more of these biological molecules.

The invention also provides kits for treating a genetic disease, which kits comprise at least an antisense oligonucleotide of the present invention, packaged in a suitable container and instructions for its use.

Other aspects and advantages of the invention will become apparent to those skilled in the art from a review of the ensuing description, which proceeds with reference to the following figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 Schematic representation of motifs and domains involved in exon recognition, intron removal and the splicing process (SEQ ID NOS: 213 and 214).

FIG. 2 Diagrammatic representation of the concept of antisense oligonucleotide induced exon skipping to by-pass disease-causing mutations (not drawn to scale). The hatched box represents an exon carrying a mutation that prevents the translation of the rest of the mRNA into a protein. The solid black bar represents an antisense oligonucleotide that prevents inclusion of that exon in the mature mRNA.

FIG. 3 Gel electrophoresis showing differing efficiencies of two antisense molecules directed at exon 8 acceptor splice site. The preferred compound [H8A(-06+18)] induces strong and consistent exon skipping at a transfection concentration of 20 nanomolar in cultured normal human muscle cells. The less preferred antisense oligonucleotide [H8A(-06+14)] also induces efficient exon skipping, but at much higher concentrations. Other antisense oligonucleotides directed at exon 8 either only induced lower levels of exon skipping or no detectable skipping at all (not shown).

FIG. 4 Gel electrophoresis showing differing efficiencies of two antisense molecules directed at internal domains within exon 7, presumably exon splicing enhancers. The preferred compound [H7A(+45+67)] induces strong and consistent exon skipping at a transfection concentration of 20 nanomolar in cultured human muscle cells. The less preferred antisense oligonucleotide [H7A(+2+26)] induces only low levels of exon skipping at the higher transfection concentrations. Other antisense oligonucleotides directed at exon 7 either only induced lower levels of exon skipping or no detectable skipping at all (not shown).

FIG. 5 Gel electrophoresis showing an example of low efficiency exon 6 skipping using two non-preferred antisense molecules directed at human exon 6 donor splice site. Levels of induced exon 6 skipping are either very low [H6D(+04-21)] or almost undetectable [H6D(+18-04)]. These are examples of non-preferred antisense oligonucleotides that demonstrate that antisense oligonucleotide design plays a crucial role in the efficacy of these compounds.

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FIG. 6 Gel electrophoresis showing strong and efficient human exon 6 skipping using an antisense molecules [H6A(+69+91)] directed at an exon 6 internal domain, presumably an exon splicing enhancer. This preferred compound induces consistent exon skipping at a transfection concentration of 20 nanomolar in cultured human muscle cells.

FIG. 7 Gel electrophoresis showing strong human exon 4 skipping using an antisense molecule H4A(+13+32) directed at an exon 6 internal domain, presumably an exon splicing enhancer. This preferred compound induces strong and consistent exon skipping at a transfection concentration of 20 nanomolar in cultured human muscle cells.

FIG. 8A Gel electrophoresis showing strong human exon 12 skipping using antisense molecule H12A(+52+75) directed at exon 12 internal domain.

FIG. 8B Gel electrophoresis showing strong human exon 11 skipping using antisense molecule H11A(+75+97) directed at an exon 11 internal domain.

FIG. 9A Gel electrophoresis showing strong human exon 15 skipping using antisense molecules H15A(+48+71) and H15A(-12+19) directed at an exon 15 internal domain.

FIG. 9B Gel electrophoresis showing strong human exon 16 skipping using antisense molecules H16A(-12+19) and H16A(-06+25).

FIG. 10 Gel electrophoresis showing human exon 19/20 skipping using antisense molecules H20A(+44+71) and H20A(+149+170) directed at an exon 20 and a "cocktail" of antisense oligonucleotides H19A(+35+65, H20A(+44+71) and H20A(+149+170) directed at exons 19/20.

FIG. 11 Gel electrophoresis showing human exon 19/20 skipping using "weasels" directed at exons 19 and 20.

FIG. 12 Gel electrophoresis showing exon 22 skipping using antisense molecules H22A(+125+106), H22A(+47+69), H22A(+80+101) and H22D(+13-11) directed at exon 22.

FIG. 13 Gel electrophoresis showing exon 31 skipping using antisense molecules H31D(+01-25) and H31D(+03-22); and a "cocktail" of antisense molecules directed at exon 31.

FIG. 14 Gel electrophoresis showing exon 33 skipping using antisense molecules H33A(+30+56) and H33A(+64+88) directed at exon 33.

FIG. 15 Gel electrophoresis showing exon 35 skipping using antisense molecules H35A(+141+161), H35A(+116+135), and H35A(+24+43) and a "cocktail" of two antisense molecules, directed at exon 35.

FIG. 16 Gel electrophoresis showing exon 36 skipping using antisense molecules H32A(+49+73) and H36A(+26+50) directed at exon 36.

FIG. 17 Gel electrophoresis showing exon 37 skipping using antisense molecules H37A(+82+105) and H37A(+134+157) directed at exon 37.

FIG. 18 Gel electrophoresis showing exon 38 skipping using antisense molecule H38A(+88+112) directed at exon 38.

FIG. 19 Gel electrophoresis showing exon 40 skipping using antisense molecule H40A(-05+17) directed at exon 40.

FIG. 20 Gel electrophoresis showing exon 42 skipping using antisense molecule H42A(-04+23) directed at exon 42.

FIG. 21 Gel electrophoresis showing exon 46 skipping using antisense molecule H46A(+86+115) directed at exon 46.

FIG. 22 Gel electrophoresis showing exon 51, exon 52 and exon 53 skipping using various antisense molecules directed at exons 51, 52 and 53, respectively. A "cocktail" of antisense molecules is also shown directed at exon 53.

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BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

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TABLE 1A

Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
1	H8A(-06+18)	GAU AGG UGG UAU CAA CAU CUG UAA
2	H8A (-03+18)	GAU AGG UGG UAU CAA CAU CUG
3	H8A(-07+18)	GAU AGG UGG UAU CAA CAU CUG UAA G
4	H8A(-06+14)	GGU GGU AUC AAC AUC UGU AA
5	H8A(-10+10)	GUA UCA ACA UCU GUA AGC AC
6	H7A(+45+67)	UGC AUG UUC CAG UCG UUG UGU GG
7	H7A(+02+26)	CAC UAU UCC AGU CAA AUA GGU CUG G
8	H7D(+15-10)	AUU UAC CAA CCU UCA GGA UCG AGU A
9	H7A(-18+03)	GGC CUA AAA CAC AUA CAC AUA
10	C6A(-10+10)	CAU UUU UGA CCU ACA UGU GG
11	C6A(-14+06)	UUU GAC CUA CAU GUG GAA AG
12	C6A(-14+12)	UAC AUU UUU GAC CUA CAU GUG GAA AG
13	C6A(-13+09)	AUU UUU GAC CUA CAU GGG AAA G
14	CH6A(+69+91)	UAC GAG UUG AUU GUC GGA CCC AG
15	C6D(+12-13)	GUG GUC UCC UUA CCU AUG ACU GUG G
16	C6D(+06-11)	GGU CUC CUU ACC UAU GA
17	H6D(+04-21)	UGU CUC AGU AAU CUU CUU ACC UAU
18	H6D(+18-04)	UCU UAC CUA UGA CUA UGG AUG AGA
19	H4A(+13+32)	GCA UGA ACU CUU GUG GAU CC
20	H4D(+04-16)	CCA GGG UAC UAC UUA CAU UA
21	H4D(-24-44)	AUC GUG UGU CAC AGC AUC CAG
22	H4A(+11+40)	UGU UCA GGG CAU GAA CUC UUG UGG AUC CUU
23	H3A(+30+60)	UAG GAG GCG CCU CCC AUC CUG UAG GUC ACU G
24	H3A(+35+65)	AGG UCU AGG AGG CGC CUC CCA UCC UGU AGG U
25	H3A(+30+54)	GCG CCU CCC AUC CUG UAG GUC ACU G
26	H3D(+46-21)	CUU CGA GGA GGU CUA GGA GGC GCC UC
27	H3A(+30+50)	CUC CCA UCC UGU AGG UCA CUG
28	H3D(+19-03)	UAC CAG UUU UUG CCC UGU CAG G
29	H3A(-06+20)	UCA AUA UGC UGC UUC CCA AAC UGA AA
30	H3A(+37+61)	CUA GGA GGC GCC UCC CAU CCU GUA G

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TABLE 1A-continued

Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
31	H5A(+20+50)	UUA UGA UUU CCA UCU ACG AUG UCA GUA CUU C
32	H5D(+25-05)	CUU ACC UGC CAG UGG AGG AUU AUA UUC CAA A
33	H5D(+10-15)	CAU CAG GAU UCU UAC CUG CCA GUG G
34	H5A(+10+34)	CGA UGU CAG UAC UUC CAA UAU UCA C
35	H5D(-04-21)	ACC AUU CAU CAG GAU UCU
36	H5D(+16-02)	ACC UGC CAG UGG AGG AUU
37	H5A(-07+20)	CCA AUA UUC ACU AAA UCA ACC UGU UAA
38	H5D(+18-12)	CAG GAU UGU UAC CUG CCA GUG GAG GAU UAU
39	H5A(+05+35)	ACG AUG UCA GUA CUU CCA AUA UUC ACU AAA U
40	H5A(+15+45)	AUU UCC AUC UAC GAU GUC AGU ACU UCC AAU A
41	H10A(-05+16)	CAG GAG CUU CCA AAU GCU GCA
42	H10A(-05+24)	CUU GUC UUC AGG AGC UUC CAA AUG CUG CA
43	H10A(+98+119)	UCC UCA GCA GAA AGA AGC CAC G
44	H10A(+130+149)	UUA GAA AUC UCU CCU UGU GC
45	H10A(-33-14)	UAA AUU GGG UGU UAC ACA AU
46	H11D(+26+49)	CCC UGA GGC AUU CCC AUC UUG AAU
47	H11D(+11-09)	AGG ACU UAC UUG CUU UGU UU
48	H11A(+118+140)	CUU GAA UUU AGG AGA UUC AUC UG
49	H11A(+75+97)	CAU CUU CUG AUA AUU UUC CUG UU
50	H12A(+52+75)	UCU UCU GUU UUU GUU AGC CAG UCA
51	H12A(-10+10)	UCU AUG UAA ACU GAA AAU UU
52	H12A(+11+30)	UUC UGG AGA UCC AUU AAA AC
53	H13A(+77+100)	CAG CAG UUG CGU GAU CUC CAC UAG
54	H13A(+55+75)	UUC AUC AAC UAC CAC CAC CAU
55	H13D(+06-19)	CUA AGC AAA AUA AUC UGA CCU UAA G
56	H14A(+37+64)	CUU GUA AAA GAA CCC AGC GGU CUU CUG U
57	H14A(+14+35)	CAU CUA CAG AUG UUU GCC CAU C
58	H14A(+51+73)	GAA GGA UGU CUU GUA AAA GAA CC
59	H14D(-02+18)	ACC UGU UCU UCA GUA AGA CG
60	H14D(+14-10)	CAU GAC ACA CCU GUU CUU CAG UAA
61	H14A(+61+80)	CAU UUG AGA AGG AUG UCU UG
62	H14A(-12+12)	AUC UCC CAA UAC CUG GAG AAG AGA

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TABLE 1A-continued

Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
63	H15A(-12+19)	GCC AUG CAC UAA AAA GGC ACU GCA AGA CAU U
64	H15A(+48+71)	UCU UUA AAG CCA GUU GUG UGA AUC
65	H15A(+08+28)	UUU CUG AAA GCC AUG CAC UAA
66	H15D(+17-08)	GUA CAU ACG GCC AGU UUU UGA AGA C
67	H16A(-12+19)	CUA GAU CCG CUU UUA AAA CCU GUU AAA ACA A
68	H16A(-06+25)	UCU UUU CUA GAU CCG CUU UUA AAA CCU GUU A
69	H16A(-06+19)	CUA GAU CCG CUU UUA AAA CCU GUU A
70	H16A(+87+109)	CCG UCU UCU GGG UCA CUG ACU UA
71	H16A(-07+19)	CUA GAU CCG CUU UUA AAA CCU GUU AA
72	H16A(-07+13)	CCG CUU UUA AAA CCU GUU AA
73	H16A(+12+37)	UGG AUU GCU UUU UCU UUU CUA GAU CC
74	H16A(+92+116)	CAU GCU UCC GUC UUC UGG GUC ACU G
75	H16A(+45+67)	G AUC UUG UUU GAG UGA AUA CAG U
76	H16A(+105+126)	GUU AUC CAG CCA UGC UUC CGU C
77	H16D(+05-20)	UGA UAA UUG GUA UCA CUA ACC UGU G
78	H16D(+12-11)	GUA UCA CUA ACC UGU GCU GUA C
79	H19A(+35+53)	CUG CUG GCA UCU UGC AGU U
80	H19A(+35+65)	GCC UGA GCU GAU CUG CUG GCA UCU UGC AGU U
81	H20A(+44+71)	CUG GCA GAA UUC GAU CCA CCG GCU GUU C
82	H20A(+147+168)	CAG CAG UAG UUG UCA UCU GCU C
83	H20A(+185+203)	UGA UGG GGU GGU GGG UUG G
84	H20A(-08+17)	AUC UGC AUU AAC ACC CUC UAG AAA G
85	H20A(+30+53)	CCG GCU GUU CAG UUG UUC UGA GGC
86	H20A(-11+17)	AUC UGC AUU AAC ACC CUC UAG AAA GAA A
87	H20D(+08-20)	GAA GGA GAA GAG AUU CUU ACC UUA CAA A
88	H20A(+44+63)	AUU CGA UCC ACC GGC UGU UC
89	H20A(+149+168)	CAG CAG UAG UUG UCA UCU GC
90	H21A(-06+16)	GCC GGU UGA CUU CAU CCU GUG C
91	H21A(+85+106)	CUG CAU CCA GGA ACA UGG GUC C
92	H21A(+85+108)	GUC UGC AUC CAG GAA CAU GGG UC
93	H21A(+08+31)	GUU GAA GAU CUG AUA GCC GGU UGA
94	H21D(+18-07)	UAC UUA CUG UCU GUA GCU CUU UCU
95	H22A(+22+45)	CAC UCA UGG UCU CCU GAU AGC GCA

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TABLE 1A-continued

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Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
96	H22A(+125+106)	CUG CAA UUC CCC GAG UCU CUG C
97	H22A(+47+69)	ACU GCU GGA CCC AUG UCC UGA UG
98	H22A(+80+101)	CUA AGU UGA GGU AUG GAG AGU
99	H22D(+13-11)	UAU UCA CAG ACC UGC AAU UCC CC
100	H23A(+34+59)	ACA GUG GUG CUG AGA UAG UAU AGG CC
101	H23A(+18+39)	UAG GCC ACU UUG UUG CUC UUG C
102	H23A(+72+90)	UUC AGA GGG CGC UUU CUU C
103	H24A(+48+70)	GGG CAG GCC AUU CCU CCU UCA GA
104	H24A(-02+22)	UCU UCA GGG UUU GUA UGU GAU UCU
105	H25A(+9+36)	CUG GGC UGA AUU GUC UGA AUA UCA CUG
106	H25A(+131+156)	CUG UUG GCA CAU GUG AUC CCA CUG AG
107	H25D(+16-08)	GUC UAU ACC UGU UGG CAC AUG UGA
108	H26A(+132+156)	UGC UUU CUG UAA UUC AUC UGG AGU U
109	H26A(-07+19)	CCU CCU UUC UGG CAU AGA CCU UCC AC
110	H26A(+68+92)	UGU GUC AUC CAU UCG UGC AUC UCU G
111	H27A(+82+106)	UUA AGG CCU CUU GUG CUA CAG GUG G
112	H27A(-4+19)	GGG GCU CUU CUU UAG CUC UCU GA
113	H27D(+19-03)	GAC UUC CAA AGU CUU GCA UUU C
114	H28A(-05+19)	GCC AAC AUG CCC AAA CUU CCU AAG
115	H28A(+99+124)	CAG AGA UUU CCU CAG CUC CGC CAG GA
116	H28D(+16-05)	CUU ACA UCU AGC ACC UCA GAG
117	H29A(+57+81)	UCC GCC AUC UGU UAG GGU CUG UGC C
118	H29A(+18+42)	AUU UGG GUU AUC CUC UGA AUG UCG C
119	H29D(+17-05)	CAU ACC UCU UCA UGU AGU UCC C
120	H30A(+122+147)	CAU UUG AGC UGC GUC CAC CUU GUC UG
121	H30A(+25+50)	UCC UGG GCA GAC UGG AUG CUC UGU UC
122	H30D(+19-04)	UUG CCU GGG CUU CCU GAG GCA UU
123	H31D(+06-18)	UUC UGA AAU AAC AUA UAC CUG UGC
124	H31D(+03-22)	UAG UUU CUG AAA UAA CAU AUA CCU G
125	H31A(+05+25)	GAC UUG UCA AAU CAG AUU GGA
126	H31D(+04-20)	GUU UCU GAA AUA ACA UAU ACC UGU
127	H32D(+04-16)	CAC CAG AAA UAC AUA CCA CA
128	H32A(+151+170)	CAA UGA UUU AGC UGU GAC UG
129	H32A(+10+32)	CGA AAC UUC AUG GAG ACA UCU UG

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TABLE 1A-continued

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Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
130	H32A(+49+73)	CUU GUA GAC GCU GCU CAA AAU UGG C
131	H33D(+09-11)	CAU GCA CAC ACC UUU GCU CC
132	H33A(+53+76)	UCU GUA CAA UCU GAC GUC CAG UCU
133	H33A(+30+56)	GUC UUU AUC ACC AUU UCC ACU UCA GAC
134	H33A(+64+88)	CCG UCU GCU UUU UCU GUA CAA UCU G
135	H34A(+83+104)	UCC AUA UCU GUA GCU GCC AGC C
136	H34A(+143+165)	CCA GGC AAC UUC AGA AUC CAA AU
137	H34A(-20+10)	UUU CUG UUA CCU GAA AAG AAU UAU AAU GAA
138	H34A(+46+70)	CAU UCA UUU CCU UUC GCA UCU UAC G
139	H34A(+95+120)	UGA UCU CUU UGU CAA UUC CAU AUC UG
140	H34D(+10-20)	UUC AGU GAU AUA GGU UUU ACC UUU CCC CAG
141	H34A(+72+96)	CUG UAG CUG CCA GCC AUU CUG UCA AG
142	H35A(+141+161)	UCU UCU GCU CGG GAG GUG ACA
143	H35A(+116+135)	CCA GUU ACU AUU CAG AAG AC
144	H35A(+24+43)	UCU UCA GGU GCA CCU UCU GU
145	H36A(+26+50)	UGU GAU GUG GUC CAC AUU CUG GUC A
146	H36A(-02+18)	CCA UGU GUU UCU GGU AUU CC
147	H37A(+26+50)	CGU GUA GAG UCC ACC UUU GGG CGU A
148	H37A(+82+105)	UAC UAA UUU CCU GCA GUG GUC ACC
149	H37A(+134+157)	UUC UGU GUG AAA UGG CUG CAA AUC
150	H38A(-01+19)	CCU UCA AAG GAA UGG AGG CC
151	H38A(+59+83)	UGC UGA AUU UCA GCC UCC AGU GGU U
152	H38A(+88+112)	UGA AGU CUU CCU CUU UCA GAU UCA C
153	H39A(+62+85)	CUG GCU UUC UCU CAU CUG UGA UUC
154	H39A(+39+58)	GUU GUA AGU UGU CUC CUC UU
155	H39A(+102+121)	UUG UCU GUA ACA GCU GCU GU
156	H39D(+10-10)	GCU CUA AUA CCU UGA GAG CA
157	H40A(-05+17)	CUU UGA GAC CUC AAA UCC UGU U
158	H40A(+129+153)	CUU UAU UUU CCU UUC AUC UCU GGG C
159	H42A(-04+23)	AUC GUU UCU UCA CGG ACA GUG UGC UGG
160	H42A(+86+109)	GGG CUU GUG AGA CAU GAG UGA UUU
161	H42D(+19-02)	A CCU UCA GAG GAC UCC UCU UGC
162	H43D(+10-15)	UAU GUG UUA CCU ACC CUU GUC GGU C
163	H43A(+101+120)	GGA GAG AGC UUC CUG UAG CU

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TABLE 1A-continued

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Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
164	H43A(+78+100)	UCA CCC UUU CCA CAG GCG UUG CA
165	H44A(+85+104)	UUU GUG UCU UUC UGA GAA AC
166	H44D(+10-10)	AAA GAC UUA CCU UAA GAU AC
167	H44A(-06+14)	AUC UGU CAA AUC GCC UGC AG
168	H46D(+16-04)	UUA CCU UGA CUU GCU CAA GC
169	H46A(+90+109)	UCC AGG UUC AAG UGG GAU AC
170	H47A(+76+100)	GCU CUU CUG GGC UUA UGG GAG CAC U
171	H47D(+25-02)	ACC UUU AUC CAC UGG AGA UUU GUC UGC
172	H47A(-9+12)	UUC CAC CAG UAA CUG AAA CAG
173	H50A(+02+30)	CCA CUC AGA GCU CAG AUC UUC UAA CUU CC
174	H50A(+07+33)	CUU CCA CUC AGA GCU CAG AUC UUC UAA
175	H50D(+07-18)	GGG AUC CAG UAU ACU UAC AGG CUC C
176	H51A(-01+25)	ACC AGA GUA ACA GUC UGA GUA GGA GC
177	H51D(+16-07)	CUC AUA CCU UCU GCU UGA UGA UC
178	H51A(+111 +134)	UUC UGU CCA AGC CCG GUU GAA AUC
179	H51A(+61+90)	ACA UCA AGG AAG AUG GCA UUU CUA GUU UGG
180	H51A(+66+90)	ACA UCA AGG AAG AUG GCA UUU CUA G
181	H51A(+66+95)	CUC CAA CAU CAA GGA AGA UGG CAU UUC UAG
182	H51D(+08-17)	AUC AUU UUU UCU CAU ACC UUC UGC U
183	H51A/D(+08-17) & (-15+)	AUC AUU UUU UCU CAU ACC UUC UGC UAG GAG CUA AAA
184	H51A(+175+195)	CAC CCA CCA UCA CCC UCU GUG
185	H51A(+199+220)	AUC AUC UCG UUG AUA UCC UCA A
186	H52A(-07+14)	UCC UGC AUU GUU GCC UGU AAG
187	H52A(+12+41)	UCC AAC UGG GGA CGC CUC UGU UCC AAA UCC
188	H52A(+17+37)	ACU GGG GAC GCC UCU GUU CCA
189	H52A(+93+112)	CCG UAA UGA UUG UUC UAG CC
190	H52D(+05-15)	UGU UAA AAA ACU UAC UUC GA
191	H53A(+45+69)	CAU UCA ACU GUU GCC UCC GGU UCU G

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TABLE 1A-continued

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Description of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA. Since these 2'-O-methyl antisense oligonucleotides are more RNA-like, U represents uracil. With other antisense chemistries such as peptide nucleic acids or morpholinos, these U bases may be shown as "T".

SEQ ID SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
192 H53A(+39+62)	CUG UUG CCU CCG GUU CUG AAG GUG
193 H53A(+39+69)	CAU UCA ACU GUU GCC UCC GGU UCU GAA GGU G
194 H53D(+14-07)	UAC UAA CCU UGG UUU CUG UGA
195 H53A(+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
196 H53A(+150+176)	UGU AUA GGG ACC CUC CUU CCA UGA CUC
197 H53D(+20-05)	CUA ACC UUG GUU UCU GUG AUU UUC U
198 H53D(+09-18)	GGU AUC UUU GAU ACU AAC CUU GGU UUC
199 H53A(-12+10)	AUU CUU UCA ACU AGA AUA AAA G
200 H53A(-07+18)	GAU UCU GAA UUC UUU CAA CUA GAA U
201 H53A(+07+26)	AUC CCA CUG AUU CUG AAU UC
202 H53A(+124+145)	UUG GCU CUG GCC UGU CCU AAG A
203 H46A(+86+115)	CUC UUU UCC AGG UUC AAG UGG GAU ACU AGC
204 H46A(+107+137)	CAA GCU UUU CUU UUA GUU GCU GCU CUU UUC C
205 H46A(-10+20)	UAU UCU UUU GUU CUU CUA GCC UGG AGA AAG
206 H46A(+50+77)	CUG CUU CCU CCA ACC AUA AAA CAA AUU C
207 H45A(-06+20)	CCA AUG CCA UCC UGG AGU UCC UGU AA
208 H45A(+91 +110)	UCC UGU AGA AUA CUG GCA UC
209 H45A(+125+151)	UGC AGA CCU CCU GCC ACC GCA GAU UCA
210 H45D(+16 -04)	CUA CCU CUU UUU UCU GUC UG
211 H45A(+71+90)	UGU UUU UGA GGA UUG CUG AA

TABLE 1B

Description of a cocktail of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA.

SEQ ID SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
81 H20A(+44+71)	CUG GCA GAA UUC GAU CCA CCG GCU
82 H20A(+147+168)	GUU C CAG CAG UAG UUG UCA UCU GCU C
80 H19A(+35+65)	GCC UGA GCU GAU CUG CUG GCA UCU
81 H20A(+44+71)	UGC
82 H20A(+147+168)	AGU U CUG GCA GAA UUC GAU CCA CCG GCU GUU C CAG CAG UAG UUG UCA UCU GCU C

TABLE 1B-continued

Description of a cocktail of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA.

SEQ ID SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
194H53D(+14-07)	UAC UAA CCU UGG UUU CUG UGA
195H53A(+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C
196H53A(+150+175)	UGU AUA GGG ACC CUC CUU CCA UGA CUC

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TABLE 1C

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Description of a "weasel" of 2'-O-methyl phosphorothioate antisense oligonucleotides that have been used to date to study induced exon skipping during the processing of the dystrophin pre-mRNA.

SEQ ID	SEQUENCE	NUCLEOTIDE SEQUENCE (5'-3')
81	H20A(+44+71) -	CUG GCA GAA UUC GAU CCA CCG GCU GUU C-
82	H20A(+147+168)	CAG CAG UAG UUG UCA UCU GCU C
80	H19A(+35+65) -	GCC UGA GCU GAU CUG CUG GCA UCU UGC
88	H20A(+44+63) -	AGU U
79	H20A(+149+168)	-AUU CGA UCC ACC GGC UGU UC-
80	H19A(+35+65) -	CUG CUG GCA UCU UGC AGU U
88	H20A(+44+63)	GCC UGA GCU GAU CUG CUG GCA UCU UGC
80	H19A(+35+65) -	AGU U
79	H20A(+149+168)	-AUU CGA UCC ACC GGC UGU UC-
80	H19A(+35+65) -	GCC UGA GCU GAU CUG CUG GCA UCU UGC
79	H20A(+149+168)	AGU U
138	H34A(+46+70) -	-CUG CUG GCA UCU UGC AGU U
139	H34A(+94+120)	CAU UCA UUU CCU UUC GCA UCU UAC G-
124	H31D(+03-22) -	UGA UCU CUU UGU CAA UUC CAU AUC UG
144	H35A(+24+43)	UAG UUU CUG AAA UAA CAU AUA CCU G-
195	H53A(+23+47) -	UU-
196	H53A(+150+175) -	UCU UCA GGU GCA CCU UCU GU
194	H53D(+14-07)	CUG AAG GUG UUC UUG UAC UUC AUC C-
212	19/20/20	AA-
		UGU AUA GGG ACC CUC CUU CCA UGA CUC-
		AA-
		UAC UAA CCU UGG UUU CUG UGA
		- Aimed at exons
		CAG CAG UAG UUG UCA UCU GCU CAA CUG
		GCA GAA UUC GAU CCA CCG GCU GUU CAA
		GCC UGA GCU GAU CUG CUC GCA UCU
		UGC AGU

DETAILED DESCRIPTION OF THE INVENTION

General

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variation and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention as described herein.

Sequence identity numbers (SEQ ID NO:) containing nucleotide and amino acid sequence information included in this specification are collected at the end of the description and have been prepared using the programme PatentIn Version 3.0. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210>1, <210>2, etc.). The length, type of sequence and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are

defined by the information provided in numeric indicator field <400> followed by the sequence identifier (e.g. <400>1, <400>2, etc.).

An antisense molecules nomenclature system was proposed and published to distinguish between the different antisense molecules (see Mann et al., (2002) *J Gen Med* 4, 644-654). This nomenclature became especially relevant when testing several slightly different antisense molecules, all directed at the same target region, as shown below:

HH#A/D(x,y).

The first letter designates the species (e.g. H: human, M: murine, C: canine) "#" designates target dystrophin exon number.

"A/D" indicates acceptor or donor splice site at the beginning and end of the exon, respectively.

(x y) represents the annealing coordinates where "-" or "+" indicate intronic or exonic sequences respectively. As an example, A(-6+18) would indicate the last 6 bases of the intron preceding the target exon and the first 18 bases of the target exon. The closest splice site would be the acceptor so these coordinates would be preceded with an "A". Describing annealing coordinates at the donor splice site could be D(+2-18) where the last 2 exonic bases and the first 18 intronic bases correspond to the annealing site of the antisense molecule. Entirely exonic annealing coordinates that would be represented by A(+65+85), that is the site between the 65th and 85th nucleotide from the start of that exon.

The entire disclosures of all publications (including patents, patent applications, journal articles, laboratory manu-

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als, books, or other documents) cited herein are hereby incorporated by reference. No admission is made that any of the references constitute prior art or are part of the common general knowledge of those working in the field to which this invention relates.

As used necessarily herein the term "derived" and "derived from" shall be taken to indicate that a specific integer may be obtained from a particular source albeit not directly from that source.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

Description of the Preferred Embodiment

When antisense molecule(s) are targeted to nucleotide sequences involved in splicing in exons within pre-mRNA sequences, normal splicing of the exon may be inhibited causing the splicing machinery to by-pass the entire mutated exon from the mature mRNA. The concept of antisense oligonucleotide induced exon skipping is shown in FIG. 2. In many genes, deletion of an entire exon would lead to the production of a non-functional protein through the loss of important functional domains or the disruption of the reading frame. In some proteins, however, it is possible to shorten the protein by deleting one or more exons, without disrupting the reading frame, from within the protein without seriously altering the biological activity of the protein. Typically, such proteins have a structural role and/or possess functional domains at their ends. The present invention describes antisense molecules capable of binding to specified dystrophin pre-mRNA targets and re-directing processing of that gene.

Antisense Molecules

According to a first aspect of the invention, there is provided antisense molecules capable of binding to a selected target to induce exon skipping. To induce exon skipping in exons of the Dystrophin gene transcript, the antisense molecules are preferably selected from the group of compounds shown in Table 1A. There is also provided a combination or "cocktail" of two or more antisense oligonucleotides capable of binding to a selected target to induce exon skipping. To induce exon skipping in exons of the Dystrophin gene transcript, the antisense molecules in a "cocktail" are preferably selected from the group of compounds shown in Table 1B. Alternatively, exon skipping may be induced by antisense oligonucleotides joined together "weasels" preferably selected from the group of compounds shown in Table 1C.

Designing antisense molecules to completely mask consensus splice sites may not necessarily generate any skipping of the targeted exon. Furthermore, the inventors have discovered that size or length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules. With some targets such as exon 19, antisense oligonucleotides as short as 12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides. In some other targets, such as murine dystrophin exon 23, antisense oligonucleotides

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only 17 residues long were able to induce more efficient skipping than another overlapping compound of 25 nucleotides.

The inventors have also discovered that there does not appear to be any standard motif that can be blocked or masked by antisense molecules to redirect splicing. In some exons, such as mouse dystrophin exon 23, the donor splice site was the most amenable to target to re-direct skipping of that exon. It should be noted that designing and testing a series of exon 23 specific antisense molecules to anneal to overlapping regions of the donor splice site showed considerable variation in the efficacy of induced exon skipping. As reported in Mann et al., (2002) there was a significant variation in the efficiency of bypassing the nonsense mutation depending upon antisense oligonucleotide annealing ("Improved antisense oligonucleotide induced exon skipping in the mdx mouse model of muscular dystrophy". *J Gen Med* 4: 644-654). Targeting the acceptor site of exon 23 or several internal domains was not found to induce any consistent exon 23 skipping.

In other exons targeted for removal, masking the donor splice site did not induce any exon skipping. However, by directing antisense molecules to the acceptor splice site (human exon 8 as discussed below), strong and sustained exon skipping was induced. It should be noted that removal of human exon 8 was tightly linked with the co-removal of exon 9. There is no strong sequence homology between the exon 8 antisense oligonucleotides and corresponding regions of exon 9 so it does not appear to be a matter of cross reaction. Rather the splicing of these two exons is inextricably linked. This is not an isolated instance as the same effect is observed in canine cells where targeting exon 8 for removal also resulted in the skipping of exon 9. Targeting exon 23 for removal in the mouse dystrophin pre-mRNA also results in the frequent removal of exon 22 as well. This effect occurs in a dose dependent manner and also indicates close coordinated processing of 2 adjacent exons.

In other targeted exons, antisense molecules directed at the donor or acceptor splice sites did not induce exon skipping while annealing antisense molecules to intra-exonic regions (i.e. exon splicing enhancers within human dystrophin exon 6) was most efficient at inducing exon skipping. Some exons, both mouse and human exon 19 for example, are readily skipped by targeting antisense molecules to a variety of motifs. That is, targeted exon skipping is induced after using antisense oligonucleotides to mask donor and acceptor splice sites or exon splicing enhancers.

To identify and select antisense oligonucleotides suitable for use in the modulation of exon skipping, a nucleic acid sequence whose function is to be modulated must first be identified. This may be, for example, a gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. Within the context of the present invention, preferred target site(s) are those involved in mRNA splicing (i.e. splice donor sites, splice acceptor sites, or exonic splicing enhancer elements). Splicing branch points and exon recognition sequences or splice enhancers are also potential target sites for modulation of mRNA splicing.

Preferably, the present invention aims to provide antisense molecules capable of binding to a selected target in the dystrophin pre-mRNA to induce efficient and consistent exon skipping. Duchenne muscular dystrophy arises from mutations that preclude the synthesis of a functional dystrophin gene product. These Duchenne muscular dystrophy gene defects are typically nonsense mutations or genomic

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rearrangements such as deletions, duplications or micro-deletions or insertions that disrupt the reading frame. As the human dystrophin gene is a large and complex gene with the 79 exons being spliced together to generate a mature mRNA with an open reading frame of approximately 11,000 bases, there are many positions where these mutations can occur. Consequently, a comprehensive antisense oligonucleotide based therapy to address many of the different disease-causing mutations in the dystrophin gene will require that many exons can be targeted for removal during the splicing process.

Within the context of the present invention, preferred target site(s) are those involved in mRNA splicing (i.e. splice donor sites, splice acceptor sites or exonic splicing enhancer elements). Splicing branch points and exon recognition sequences or splice enhancers are also potential target sites for modulation of mRNA splicing.

The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridisable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense molecule need not be 100% complementary to that of its target sequence to be specifically hybridisable. An antisense molecule is specifically hybridisable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

While the above method may be used to select antisense molecules capable of deleting any exon from within a protein that is capable of being shortened without affecting its biological function, the exon deletion should not lead to a reading frame shift in the shortened transcribed mRNA. Thus, if in a linear sequence of three exons the end of the first exon encodes two of three nucleotides in a codon and the next exon is deleted then the third exon in the linear sequence must start with a single nucleotide that is capable of completing the nucleotide triplet for a codon. If the third exon does not commence with a single nucleotide there will be a reading frame shift that would lead to the generation of truncated or a non-functional protein.

It will be appreciated that the codon arrangements at the end of exons in structural proteins may not always break at the end of a codon, consequently there may be a need to delete more than one exon from the pre-mRNA to ensure in-frame reading of the mRNA. In such circumstances, a plurality of antisense oligonucleotides may need to be selected by the method of the invention wherein each is directed to a different region responsible for inducing splicing in the exons that are to be deleted.

The length of an antisense molecule may vary so long as it is capable of binding selectively to the intended location within the pre-mRNA molecule. The length of such sequences can be determined in accordance with selection procedures described herein. Generally, the antisense molecule will be from about 10 nucleotides in length up to about 50 nucleotides in length. It will be appreciated however that

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any length of nucleotides within this range may be used in the method. Preferably, the length of the antisense molecule is between 17 to 30 nucleotides in length.

In order to determine which exons can be connected in a dystrophin gene, reference should be made to an exon boundary map. Connection of one exon with another is based on the exons possessing the same number at the 3' border as is present at the 5' border of the exon to which it is being connected. Therefore, if exon 7 were deleted, exon 6 must connect to either exons 12 or 18 to maintain the reading frame. Thus, antisense oligonucleotides would need to be selected which redirected splicing for exons 7 to 11 in the first instance or exons 7 to 17 in the second instance. Another and somewhat simpler approach to restore the reading frame around an exon 7 deletion would be to remove the two flanking exons. Induction of exons 6 and 8 skipping should result in an in-frame transcript with the splicing of exons 5 to 9. In practise however, targeting exon 8 for removal from the pre-mRNA results in the co-removal of exon 9 so the resultant transcript would have exon 5 joined to exon 10. The inclusion or exclusion of exon 9 does not alter the reading frame. Once the antisense molecules to be tested have been identified, they are prepared according to standard techniques known in the art. The most common method for producing antisense molecules is the methylation of the 2' hydroxyribose position and the incorporation of a phosphorothioate backbone produces molecules that superficially resemble RNA but that are much more resistant to nuclease degradation.

To avoid degradation of pre-mRNA during duplex formation with the antisense molecules, the antisense molecules used in the method may be adapted to minimise or prevent cleavage by endogenous RNase H. This property is highly preferred as the treatment of the RNA with the unmethylated oligonucleotides either intracellularly or in crude extracts that contain RNase H leads to degradation of the pre-mRNA: antisense oligonucleotide duplexes. Any form of modified antisense molecules that is capable of bypassing or not inducing such degradation may be used in the present method. An example of antisense molecules which when duplexed with RNA are not cleaved by cellular RNase H is 2'-O-methyl derivatives. 2'-O-methyl-oligoribonucleotides are very stable in a cellular environment and in animal tissues, and their duplexes with RNA have higher T_m values than their ribo- or deoxyribo-counterparts.

Antisense molecules that do not activate RNase H can be made in accordance with known techniques (see, e.g., U.S. Pat. No. 5,149,797). Such antisense molecules, which may be deoxyribonucleotide or ribonucleotide sequences, simply contain any structural modification which sterically hinders or prevents binding of RNase H to a duplex molecule containing the oligonucleotide as one member thereof, which structural modification does not substantially hinder or disrupt duplex formation. Because the portions of the oligonucleotide involved in duplex formation are substantially different from those portions involved in RNase H binding thereto, numerous antisense molecules that do not activate RNase H are available. For example, such antisense molecules may be oligonucleotides wherein at least one, or all, of the inter-nucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphorothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues may be modified as described. In another non-limiting example, such antisense molecules are molecules wherein at least one, or all, of the nucleotides contain a 2' lower alkyl

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moiety (e.g., C₁-C₄, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl). For example, every other one of the nucleotides may be modified as described.

While antisense oligonucleotides are a preferred form of the antisense molecules, the present invention comprehends other oligomeric antisense molecules, including but not limited to oligonucleotide mimetics such as are described below.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural inter-nucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their inter-nucleoside backbone can also be considered to be oligonucleosides.

In other preferred oligonucleotide mimetics, both the sugar and the inter-nucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. Certain nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds that are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense molecules, particularly oligonucle-

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otides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the increased resistance to nuclease degradation, increased cellular uptake, and an additional region for increased binding affinity for the target nucleic acid.

Methods of Manufacturing Antisense Molecules

The antisense molecules used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Pat. No. 4,458,066.

Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives. In one such automated embodiment, diethyl-phosphoramidites are used as starting materials and may be synthesized as described by Beaucage, et al., (1981) *Tetrahedron Letters*, 22:1859-1862.

The antisense molecules of the invention are synthesized in vitro and do not include antisense compositions of biological origin, or genetic vector constructs designed to direct the in vivo synthesis of antisense molecules. The molecules of the invention may also be mixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption.

Therapeutic Agents

The present invention also can be used as a prophylactic or therapeutic, which may be utilized for the purpose of treatment of a genetic disease.

Accordingly, in one embodiment the present invention provides antisense molecules that bind to a selected target in the dystrophin pre-mRNA to induce efficient and consistent exon skipping described herein in a therapeutically effective amount admixed with a pharmaceutically acceptable carrier, diluent, or excipient.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similarly untoward reaction, such as gastric upset and the like, when administered to a patient. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in Martin, *Remington's Pharmaceutical Sciences*, 18th Ed., Mack Publishing Co., Easton, Pa., (1990).

In a more specific form of the invention there are provided pharmaceutical compositions comprising therapeutically effective amounts of an antisense molecule together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength and additives such as detergents and solubilizing agents (e.g.,

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Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). The material may be incorporated into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hylauronic acid may also be used. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Martin, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 that are herein incorporated by reference. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilised form.

It will be appreciated that pharmaceutical compositions provided according to the present invention may be administered by any means known in the art. Preferably, the pharmaceutical compositions for administration are administered by injection, orally, or by the pulmonary, or nasal route. The antisense molecules are more preferably delivered by intravenous, intra-arterial, intraperitoneal, intramuscular, or subcutaneous routes of administration.

Antisense Molecule Based Therapy

Also addressed by the present invention is the use of antisense molecules of the present invention, for manufacture of a medicament for modulation of a genetic disease.

The delivery of a therapeutically useful amount of antisense molecules may be achieved by methods previously published. For example, intracellular delivery of the antisense molecule may be via a composition comprising an admixture of the antisense molecule and an effective amount of a block copolymer. An example of this method is described in US patent application US 20040248833.

Other methods of delivery of antisense molecules to the nucleus are described in Mann C J et al., (2001) [*"Antisense-induced exon skipping and the synthesis of dystrophin in the mdx mouse"*]. *Proc., Natl. Acad. Science*, 98(1) 42-47J and in Gebiski et al., (2003). *Human Molecular Genetics*, 12(15): 1801-1811.

A method for introducing a nucleic acid molecule into a cell by way of an expression vector either as naked DNA or complexed to lipid carriers, is described in U.S. Pat. No. 6,806,084.

It may be desirable to deliver the antisense molecule in a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes or liposome formulations.

Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. These formulations may have net cationic, anionic or neutral charge characteristics and are useful characteristics with in vitro, in vivo and ex vivo delivery methods. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, and DNA can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, 6:77, 1981).

In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the antisense molecule of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous

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contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., *Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Alternatively, the antisense construct may be combined with other pharmaceutically acceptable carriers or diluents to produce a pharmaceutical composition. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, intraocular, oral or transdermal administration.

The routes of administration described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and any dosage for any particular animal and condition. Multiple approaches for introducing functional new genetic material into cells, both in vitro and in vivo have been attempted (Friedmann (1989) *Science*, 244:1275-1280).

These approaches include integration of the gene to be expressed into modified retroviruses (Friedmann (1989) *supra*; Rosenberg (1991) *Cancer Research* 51(18), suppl.: 5074S-5079S); integration into non-retrovirus vectors (Rosenfeld, et al. (1992) *Cell*, 68:143-155; Rosenfeld, et al. (1991) *Science*, 252:431-434); or delivery of a transgene linked to a heterologous promoter-enhancer element via liposomes (Friedmann (1989), *supra*; Brigham, et al. (1989) *Am. J. Med. Sci.*, 298:278-281; Nabel, et al. (1990) *Science*, 249:1285-1288; Hazinski, et al. (1991) *Am. J. Resp. Cell Molec. Biol.*, 4:206-209; and Wang and Huang (1987) *Proc. Natl. Acad. Sci. (USA)*, 84:7851-7855); coupled to ligand-specific, cation-based transport systems (Wu and Wu (1988) *J. Biol. Chem.*, 263:14621-14624) or the use of naked DNA, expression vectors (Nabel et al. (1990), *supra*); Wolff et al. (1990) *Science*, 247:1465-1468). Direct injection of transgenes into tissue produces only localized expression (Rosenfeld (1992) *supra*); Rosenfeld et al. (1991) *supra*; Brigham et al. (1989) *supra*; Nabel (1990) *supra*; and Hazinski et al. (1991) *supra*). The Brigham et al. group (*Am. J. Med. Sci.* (1989) 298:278-281 and *Clinical Research* (1991) 39 (abstract)) have reported in vivo transfection only of lungs of mice following either intravenous or intratracheal administration of a DNA liposome complex. An example of a review article of human gene therapy procedures is: Anderson, *Science* (1992) 256:808-813.

The antisense molecules of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such pro-drugs, and other bioequivalents.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

For oligonucleotides, preferred examples of pharmaceutically acceptable salts include but are not limited to (a) salts

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formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, (including by nebulizer, intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient (s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Kits of the Invention

The invention also provides kits for treatment of a patient with a genetic disease which kit comprises at least an antisense molecule, packaged in a suitable container, together with instructions for its use.

In a preferred embodiment, the kits will contain at least one antisense molecule as shown in Table 1A, or a cocktail of antisense molecules as shown in Table 1B or a "weasel" compound as shown in Table 1C. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Those of ordinary skill in the field should appreciate that applications of the above method has wide application for identifying antisense molecules suitable for use in the treatment of many other diseases.

EXAMPLES

The following Examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these Examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. The references cited herein are expressly incorporated by reference.

Methods of molecular cloning, immunology and protein chemistry, which are not explicitly described in the following examples, are reported in the literature and are known by those skilled in the art. General texts that described conventional molecular biology, microbiology, and recombinant

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DNA techniques within the skill of the art, included, for example: Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Glover et al., *DNA Cloning: A Practical Approach*, Volumes I and II, MRL Press, Ltd., Oxford, U. K. (1985); and Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K. *Current Protocols in Molecular Biology*. Greene Publishing Associates/Wiley Intersciences, New York (2002).

Determining Induced Exon Skipping in Human Muscle Cells

Attempts by the inventors to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies.

These empirical studies involved the use of computer programs to identify motifs potentially involved in the splicing process. Other computer programs were also used to identify regions of the pre-mRNA which may not have had extensive secondary structure and therefore potential sites for annealing of antisense molecules. Neither of these approaches proved completely reliable in designing antisense oligonucleotides for reliable and efficient induction of exon skipping.

Annealing sites on the human dystrophin pre-mRNA were selected for examination, initially based upon known or predicted motifs or regions involved in splicing. 20ME antisense oligonucleotides were designed to be complementary to the target sequences under investigation and were synthesised on an Expedite 8909 Nucleic Acid Synthesiser. Upon completion of synthesis, the oligonucleotides were cleaved from the support column and de-protected in ammonium hydroxide before being desalted. The quality of the oligonucleotide synthesis was monitored by the intensity of the trityl signals upon each deprotection step during the synthesis as detected in the synthesis log. The concentration of the antisense oligonucleotide was estimated by measuring the absorbance of a diluted aliquot at 260 nm.

Specified amounts of the antisense molecules were then tested for their ability to induce exon skipping in an in vitro assay, as described below.

Briefly, normal primary myoblast cultures were prepared from human muscle biopsies obtained after informed consent. The cells were propagated and allowed to differentiate into myotubes using standard culturing techniques. The cells were then transfected with the antisense oligonucleotides by delivery of the oligonucleotides to the cells as cationic lipoplexes, mixtures of antisense molecules or cationic liposome preparations.

The cells were then allowed to grow for another 24 hours, after which total RNA was extracted and molecular analysis commenced. Reverse transcriptase amplification (RT-PCR) was undertaken to study the targeted regions of the dystrophin pre-mRNA or induced exonic re-arrangements.

For example, in the testing of an antisense molecule for inducing exon 19 skipping the RT-PCR test scanned several exons to detect involvement of any adjacent exons. For example, when inducing skipping of exon 19, RT-PCR was carried out with primers that amplified across exons 17 and 21. Amplifications of even larger products in this area (i.e. exons 13-26) were also carried out to ensure that there was

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minimal amplification bias for the shorter induced skipped transcript. Shorter or exon skipped products tend to be amplified more efficiently and may bias the estimated of the normal and induced transcript.

The sizes of the amplification reaction products were estimated on an agarose gel and compared against appropriate size standards. The final confirmation of identity of these products was carried out by direct DNA sequencing to establish that the correct or expected exon junctions have been maintained.

Once efficient exon skipping had been induced with one antisense molecule, subsequent overlapping antisense molecules may be synthesized and then evaluated in the assay as described above. Our definition of an efficient antisense molecule is one that induces strong and sustained exon skipping at transfection concentrations in the order of 300 nM or less.

Antisense Oligonucleotides Directed at Exon 8

Antisense oligonucleotides directed at exon 8 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

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efficient antisense molecules only induced exon skipping at concentrations of 300 nM and above. Therefore, we have shown that targeting of the antisense molecules to motifs involved in the splicing process plays a crucial role in the overall efficacy of that compound.

Efficacy refers to the ability to induce consistent skipping of a target exon. However, sometimes skipping of the target exons is consistently associated with a flanking exon. That is, we have found that the splicing of some exons is tightly linked. For example, in targeting exon 23 in the mouse model of muscular dystrophy with antisense molecules directed at the donor site of that exon, dystrophin transcripts missing exons 22 and 23 are frequently detected. As another example, when using an antisense molecule directed to exon 8 of the human dystrophin gene, all induced transcripts are missing both exons 8 and 9. Dystrophin transcripts missing only exon 8 are not observed.

Table 2 below discloses antisense molecule sequences that induce exon 8 (and 9) skipping.

TABLE 2

SEQ ID name	Antisense Oligonucleotide	Sequence	Ability to induce skipping
1	H8A(-06+18)	5'-GAU AGG UGG UAU CAA CAU CUG UAA	Very strong to 20 nM
2	H8A (-03+18)	5'-GAU AGG UGG UAU CAA CAU CUG	Very strong skipping to 40 nM
3	H8A(-07+18)	5'-GAU AGG UGG UAU CAA CAU CUG UAA G	Strong skipping to 40 nM
4	H8A(-06+14)	5'-GGU GGU AUC AAC AUC UGU AA	Skipping to 300 nM
5	H8A(-10+10)	5'-GUA UCA ACA UCU GUA AGC AC	Patchy/weak skipping to 100 nm

FIG. 3 shows differing efficiencies of two antisense molecules directed at exon 8 acceptor splice site. H8A(-06+18) [SEQ ID NO:1], which anneals to the last 6 bases of intron 7 and the first 18 bases of exon 8, induces substantial exon 8 and 9 skipping when delivered into cells at a concentration of 20 nM. The shorter antisense molecule, H8A(-06+14) [SEQ ID NO: 4] was only able to induce exon 8 and 9 skipping at 300 nM, a concentration some 15 fold higher than H8A(-06+18), which is the preferred antisense molecule.

This data shows that some particular antisense molecules induce efficient exon skipping while another antisense molecule, which targets a near-by or overlapping region, can be much less efficient. Titration studies show one compound is able to induce targeted exon skipping at 20 nM while the less

Antisense Oligonucleotides Directed at Exon 7

Antisense oligonucleotides directed at exon 7 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 4 shows the preferred antisense molecule, H7A(+45+67) [SEQ ID NO: 6], and another antisense molecule, H7A(+2+26) [SEQ ID NO: 7], inducing exon 7 skipping. Nested amplification products span exons 3 to 9. Additional products above the induced transcript missing exon 7 arise from amplification from carry-over outer primers from the RT-PCR as well as heteroduplex formation.

Table 3 below discloses antisense molecule sequences for induced exon 7 skipping.

TABLE 3

SEQ ID name	Antisense Oligonucleotide	Sequence	Ability to induce skipping
6	H7A(+45+67)	5'-UGC AUG UUC CAG UCG UUG UGU GG	Strong skipping to 20 nM

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TABLE 3-continued

Antisense SEQ ID name	Sequence	Ability to induce skipping
7 H7A(+02+26)	5'-CAC UAU UCC AGU CAA AUA GGU CUG G	Weak skipping at 100 nM
8 H7D(+15-10)	5'-AUU UAC CAA CCU UCA GGA UCG AGU A	Weak skipping to 300 nM
9 H7A(-18+03)	5'-GGC CUA AAA CAC AUA CAC AUA	Weak skipping to 300 nM

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Antisense Oligonucleotides Directed at Exon 6

Antisense oligonucleotides directed at exon 6 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 5 shows an example of two non-preferred antisense molecules inducing very low levels of exon 6 skipping in cultured human cells. Targeting this exon for specific removal was first undertaken during a study of the canine model using the oligonucleotides as listed in Table 4, below. Some of the human specific oligonucleotides were also evaluated, as shown in FIG. 5. In this example, both antisense molecules target the donor splice site and only induced low levels of exon 6 skipping. Both H6D(+4-21) [SEQ ID NO: 17] and H6D(+18-4) [SEQ ID NO: 18] would be regarded as non-preferred antisense molecules.

One antisense oligonucleotide that induced very efficient exon 6 skipping in the canine model, C6A(+69+91) [SEQ ID NO: 14], would anneal perfectly to the corresponding region in human dystrophin exon 6. This compound was evaluated, found to be highly efficient at inducing skipping of that target exon, as shown in FIG. 6 and is regarded as the preferred compound for induced exon 6 skipping. Table 4 below discloses antisense molecule sequences for induced exon 6 skipping.

TABLE 4

Antisense Oligo SEQ ID name	Sequence	Ability to induce skipping
10 C6A(-10+10)	5' CAU UUU UGA CCU ACA UGU GG	No skipping
11 C6A(-14+06)	5' UUU GAC CUA CAU GUG GAA AG	No skipping
12 C6A(-14+12)	5' UAC AUU UUU GAC CUA CAU GUG GAA AG	No skipping
13 C6A(-13+09)	5' AUU UUU GAC CUA CAU GGG AAA G	No skipping
14 CH6A(+69+91)	5' UAC GAG UUG AUU GUC GGA CCC AG	Strong skipping to 20 nM
15 C6D(+12-13)	5' GUG GUC UCC UUA CCU AUG ACU GUG G	Weak skipping at 300 nM
16 C6D(+06-11)	5' GGU CUC CUU ACC UAU GA	No skipping
17 H6D(+04-21)	5' UGU CUC AGU AAU CUU CUU ACC UAU	Weak skipping to 50 nM
18 H6D(+18-04)	5' UCU UAC CUA UGA CUA UGG AUG AGA	Very weak skipping to 300 nM

Antisense Oligonucleotides Directed at Exon 4

Antisense oligonucleotides directed at exon 4 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 7 shows an example of a preferred antisense molecule inducing skipping of exon 4 skipping in cultured human cells. In this example, one preferred antisense compound, H4A(+13+32) [SEQ ID NO:19], which targeted a presumed exonic splicing enhancer induced efficient exon skipping at a concentration of 20 nM while other non-preferred antisense oligonucleotides failed to induce even low levels of exon 4 skipping. Another preferred antisense molecule inducing skipping of exon 4 was H4A(+111+40) [SEQ ID NO:22], which induced efficient exon skipping at a concentration of 20 nM.

Table 5 below discloses antisense molecule sequences for inducing exon 4 skipping.

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TABLE 5

SEQAntisense ID Oligonucleotide name	Sequence	Ability to induce skipping
19 H4A(+13+32)	5' GCA UGA ACU CUU GUG GAU CC	Skipping to 20 nM
22 H4A(+11+40)	5' UGU UCA GGG CAU GAA CUC UUG UGG AUC CUU	Skipping to 20 nM
20 H4D(+04-16)	5' CCA GGG UAC UAC UUA CAU UA	No skipping
21 H4D(-24-44)	5' AUC GUG UGU CAC AGC AUC CAG	No skipping

Antisense Oligonucleotides Directed at Exon 3

Antisense oligonucleotides directed at exon 3 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

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H3A(+30+60) [SEQ ID NO:23] induced substantial exon 3 skipping when delivered into cells at a concentration of 20 nM to 600 nM. The antisense molecule, H3A(+35+65) [SEQ ID NO: 24] induced exon skipping at 300 nM.

Table 6 below discloses antisense molecule sequences that induce exon 3 skipping.

TABLE 6

Antisense SEQ ID Oligonucleotide name	Sequence	Ability to induce skipping
23 H3A(+30+60)	UAG GAG GCG CCU CCC AUC CUG UAG GUC ACU G	Moderate skipping to 20 to 600 nM
24 H3A(+35+65)	AGG UCU AGG AGG CGC CUC CCA UCC UGU AGG U	Working to 300 nM
25 H3A(+30+54)	GCG CCU CCC AUC CUG UAG GUC ACU G	Moderate 100-600 nM
26 H3D(+46-21)	CUU CGA GGA GGU CUA GGA GGC GCC UC	No skipping
27 H3A(+30+50)	CUC CCA UCC UGU AGG UCA CUG	Moderate 20-600 nM
28 H3D(+19-03)	UAC CAG UUU UUG CCC UGU CAG G	No skipping
29 H3A(-06+20)	UCA AUA UGC UGC UUUCCA AAC UGA AA	No skipping
30 H3A(+37+61)	CUA GGA GGC GCC UCC CAU CCU GUA G	No skipping

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Antisense Oligonucleotides Directed at Exon 5

Antisense oligonucleotides directed at exon 5 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H5A(+20+50) [SEQ ID NO:31] induces substantial exon 5 skipping when delivered into cells at a concentration of 100 nM. Table 7 below shows other antisense molecules tested. The majority of these antisense molecules were not as effective at exon skipping as H5A(+20+50). However, H5A(+15+45) [SEQ ID NO: 40] was able to induce exon 5 skipping at 300 nM.

Table 7 below discloses antisense molecule sequences that induce exon 5 skipping.

TABLE 7

Antisense Oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
31 H5A(+20+50)	UUA UGA UUU CCA UCU ACG AUG UCA GUA CUU C	Working to 100 nM

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TABLE 7-continued

SEQ ID name	Antisense Oligonucleotide Sequence	Ability to induce skipping
32 HSD(+25-05)	CUU ACC UGC CAG UGG AGG AUU AUA UUC CAA A	No skipping
33 HSD(+10-15)	CAU CAG GAU UCU UAC CUG CCA GUG G	Inconsistent at 300 nM
34 H5A(+10+34)	CGA UGU CAG UAC UUC CAA UAU UCA C	Very weak
35 HSD(-04-21)	ACC AUU CAU CAG GAU UCU	No skipping
36 HSD(+16-02)	ACC UGC CAG UGG AGG AUU	No skipping
37 H5A(-07+20)	CCA AUA UUC ACU AAA UCA ACC UGU UAA	No skipping
38 HSD(+18-12)	CAG GAU UCU UAC CUG CCA GUG GAG GAU UAU	No skipping
39 H5A(+05+35)	ACG AUG UCA GUA CUU CCA AUA UUC ACU AAA U	No skipping
40 H5A(+15+45)	AUU UCC AUC UAC GAU GUC AGU ACU UCC AAU A	Working to 300 nM

Antisense Oligonucleotides Directed at Exon 10

Antisense oligonucleotides directed at exon 10 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H10A(-05+16) [SEQ ID NO:41] induced substantial exon 10 skipping when delivered into cells. Table 8 below shows other antisense molecules tested. The antisense molecules ability to induce exon skipping was variable. Table 8 below discloses antisense molecule sequences that induce exon 10 skipping.

TABLE 8

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
41	H10A(-05+16)	CAG GAG CUU CCA AAU GCU GCA	Not tested
42	H10A(-05+24)	CUU GUC UUC AGG AGC UUC CAA AUG CUG CA	Not tested
43	H10A(+98+119)	UCC UCA GCA GAA AGA AGC CAC G	Not tested
44	H10A(+130+149)	UUA GAA AUC UCU CCU UGU GC	No skipping
45	H10A(-33-14)	UAA AUU GGG UGU UAC ACA AU	No skipping

Antisense Oligonucleotides Directed at Exon 11

Antisense oligonucleotides directed at exon 11 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 8B shows an example of H11A(+75+97) [SEQ ID NO:49] antisense molecule inducing exon 11 skipping in cultured human cells. H11A(+75+97) induced substantial exon 11 skipping when delivered into cells at a concentration of 5 nM. Table 9 below shows other antisense molecules tested. The antisense molecules ability to induce exon skipping was observed at 100 nM.

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TABLE 9

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
46	H11D(+26+49)	CCC UGA GGC AUU CCC AUC UUG AAU	Skipping at 100 nM
47	H11D(+11-09)	AGG ACU UAC UUG CUU UGU UU	Skipping at 100 nM
48	H11A(+118+140)	CUU GAA UUU AGG AGA UUC AUC UG	Skipping at 100 nM
49	H11A(+75+97)	CAU CUU CUG AUA AUU UUC CUG UU	Skipping at 100 nM
46	H11D(+26+49)	CCC UGA GGC AUU CCC AUC UUG AAU	Skipping at 5 nM

Antisense Oligonucleotides Directed at Exon 12

Antisense oligonucleotides directed at exon 12 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H12A(+52+75) [SEQ ID NO:50] induced substantial exon 12 skipping when delivered into cells at a concentration of 5 nM, as shown in FIG. 8A. Table 10 below shows other antisense molecules tested at a concentration range of 5, 25, 50, 100, 200 and 300 nM. The antisense molecules ability to induce exon skipping was variable.

TABLE 10

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
50	H12A(+52+75)	UCU UCU GUU UUU GUU AGC CAG UCA	Skipping at 5 nM
51	H12A(-10+10)	UCU AUG UAA ACU GAA AAU UU	Skipping at 100 nM
52	H12A(+11+30)	UUC UGG AGA UCC AUU AAA AC	No skipping

Antisense Oligonucleotides Directed at Exon 13

Antisense oligonucleotides directed at exon 13 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H13A(+77+100) [SEQ ID NO:53] induced substantial exon 13 skipping when delivered into cells at a concentration of 5 nM. Table 11 below includes two other antisense

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molecules tested at a concentration range of 5, 25, 50, 100, 200 and 300 nM. These other antisense molecules were unable to induce exon skipping.

TABLE 11

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
53	H13A(+77+100)	CAG CAG UUG CGU GAU CUC CAC UAG	Skipping at 5 nM
54	H13A(+55+75)	UUC AUC AAC UAC CAC CAC CAU	No skipping
55	H13D(+06-19)	CUA AGC AAA AUA AUC UGA CCU UAA G	No skipping

Antisense Oligonucleotides Directed at Exon 14

Antisense oligonucleotides directed at exon 14 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H14A(+37+64) [SEQ ID NO:56] induced weak exon 14 skipping when delivered into cells at a concentration of 100 nM. Table 12 below includes other antisense molecules tested at a concentration range of 5, 25, 50, 100, 200 and 300 nM. The other antisense molecules were unable to induce exon skipping at any of the concentrations tested.

TABLE 12

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
56	H14A(+37+64)	CUU GUA AAA GAA CCC AGC GGU CUU CUG U	Skipping at 100 nM
57	H14A(+14+35)	CAU CUA CAG AUG UUU GCC CAU C	No skipping
58	H14A(+51+73)	GAA GGA UGU CUU GUA AAA GAA CC	No skipping

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TABLE 12-continued

SEQ ID name	Antisense Oligonucleotide Sequence	Ability to induce skipping
59 H14D(-02+18)	ACC UGU UCU UCA GUA AGA CG	No skipping
60 H14D(+14-10)	CAU GAC ACA CCU GUU CUU CAG UAA	No skipping
61 H14A(+61 +80)	CAU UUG AGA AGG AUG UCU UG	No skipping
62 H14A(-12+12)	AUC UCC CAA UAC CUG GAG AAG AGA	No skipping

Antisense Oligonucleotides Directed at Exon 15

Antisense oligonucleotides directed at exon 15 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H15A(-12+19) [SEQ ID NO:63] and H15A(+48+71) [SEQ ID NO:64] induced substantial exon 15 skipping when delivered into cells at a concentration of 10 Nm, as shown in FIG. 9A. Table 13 below includes other antisense molecules tested at a concentration range of 5, 25, 50, 100, 200 and 300 Nm. These other antisense molecules were unable to induce exon skipping at any of the concentrations tested.

TABLE 13

SEQ ID name	Antisense Oligonucleotide Sequence	Ability to induce skipping
63 H15A(-12+19)	GCC AUG CAC UAA AAA GGC ACU GCA AGA CAU U	Skipping at 5 Nm
64 H15A(+48+71)	UCU UUA AAG CCA GUU GUG UGA AUC	Skipping at 5 Nm
65 H15A(+08+28)	UUU CUG AAA GCC AUG CAC UAA	No skipping
63 H15A(-12+19)	GCC AUG CAC UAA AAA GGC ACU GCA AGA CAU U	No skipping
66 H15D(+17-08)	GUA CAU ACG GCC AGU UUU UGA AGA C	No skipping

Antisense Oligonucleotides Directed at Exon 16

Antisense oligonucleotides directed at exon 16 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H16A(-12+19) [SEQ ID NO:67] and H16A(-06+25) [SEQ ID NO:68] induced substantial exon 16 skipping when delivered into cells at a concentration of 10 nM, as shown in FIG. 9B. Table 14 below includes other antisense molecules tested. H16A(-06+19) [SEQ ID NO:69] and H16A(+87+109) [SEQ ID NO:70] were tested at a concentration range of 5, 25, 50, 100, 200 and 300 nM. These two antisense molecules were able to induce exon skipping at 25 nM and 100 nM, respectively. Additional antisense molecules were tested at 100, 200 and 300 nM and did not result in any exon skipping.

TABLE 14

SEQ ID name	Antisense Oligonucleotide Sequence	Ability to induce skipping
67 H16A(-12+19)	CUA GAU CCG CUU UUA AAA CCU GUU AAA ACA A	Skipping at 5 nM

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TABLE 14-continued

Antisense SEQ ID name	Oligonucleotide Sequence	Ability to induce skipping
68 H16A(-06+25)	UCU UUU CUA GAU CCG CUU UUA AAA CCU GUU A	Skipping at 5 nM
69 H16A(-06+19)	CUA GAU CCG CUU UUA AAA CCU GUU A	Skipping at 25 nM
70 H16A(+87+109)	CCG UCU UCU GGG UCA CUG ACU UA	Skipping at 100 nM
71 H16A(-07+19)	CUA GAU CCG CUU UUA AAA CCU GUU AA	No skipping
72 H16A(-07+13)	CCG CUU UUA AAA CCU GUU AA	No skipping
73 H16A(+12+37)	UGG AUU GCU UUU UCU UUU CUA GAU CC	No skipping
74 H16A(+92+116)	CAU GCU UCC GUC UUC UGG GUC ACU G	No skipping
75 H16A(+45+67)	G AUC UUG UUU GAG UGA AUA CAG U	No skipping
76 H16A(+105+126)	GUU AUC CAG CCA UGC UUC CGU C	No skipping
77 H16D(+05-20)	UGA UAA UUG GUA UCA CUA ACC UGU G	No skipping
78 H16D(+12-11)	GUA UCA CUA ACC UGU GCU GUA C	No skipping

Antisense Oligonucleotides Directed at Exon 19

Antisense oligonucleotides directed at exon 19 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H19A(+35+65) [SEQ ID NO:79] induced substantial exon 19 skipping when delivered into cells at a concentration of 10 nM. This antisense molecule also showed very strong exon skipping at concentrations of 25, 50, 100, 300 and 600 nM.

FIG. 10 illustrates exon 19 and 20 skipping using a "cocktail" of antisense oligonucleotides, as tested using gel electrophoresis. It is interesting to note that it was not easy to induce exon 20 skipping using single antisense oligonucleotides H20A(+44+71) [SEQ ID NO:81] or H20A(+149+170) [SEQ ID NO:82], as illustrated in sections 2 and 3 of the gel shown in FIG. 10. Whereas, a "cocktail" of antisense oligonucleotides was more efficient as can be seen in section 4 of FIG. 10 using a "cocktail" of antisense oligonucleotides H20A(+44+71) and H20A(+149+170). When the cocktail was used to target exon 19, skipping was even stronger (see section 5, FIG. 10).

FIG. 11 illustrates gel electrophoresis results of exon 19/20 skipping using "weasels" The "weasels" were effective in skipping exons 19 and 20 at concentrations of 25, 50, 100, 300 and 600 nM. A further "weasel" sequence is shown in the last row of Table 3C. This compound should give good results.

Antisense Oligonucleotides Directed at Exon 20

Antisense oligonucleotides directed at exon 20 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

None of the antisense oligonucleotides tested induced exon 20 skipping when delivered into cells at a concentration of 10, 25, 50, 300 or 600 nM (see Table 15). Antisense molecules H20A(-11+17) [SEQ ID NO:86] and H20D(+08-20) [SEQ ID NO:87] are yet to be tested.

However, a combination or "cocktail" of H20A(+44+71) [SEQ ID NO: 81] and H20(+149+170) [SEQ ID NO:82] in a ratio of 1:1, exhibited very strong exon skipping at a concentration of 100 nM and 600 nM. Further, a combination of antisense molecules H19A(+35+65) [SEQ ID NO:79], H20A(+44+71) [SEQ ID NO:81] and H20A(+149+170) [SEQ ID NO:82] in a ratio of 2:1:1, induced very strong exon skipping at a concentration ranging from 10 nM to 600 nM.

TABLE 15

Antisense SEQ ID name	Oligonucleotide Sequence	Ability to induce skipping
81 H20A(+44+71)	CUG GCA GAA UUC GAU CCA CCG GCU GUU C	No skipping
82 H20A(+147+168)	CAG CAG UAG UUG UCA UCU GCU C	No skipping
83 H20A(+195+203)	UGA UGG GGU GGU GGG UUG G	No skipping
84 H20A(-08+17)	AUC UGC AUU AAC ACC CUC UAG AAA G	No skipping

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TABLE 15-continued

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
85	H20A(+30+53)	CCG GCU GUU CAG UUG UUC UGA GGC	No skipping
86	H20A(-11+17)	AUC UGC AUU AAC ACC CUC UAG AAA GAA A	Not tested yet
87	H20D(+08-20)	GAA GGA GAA GAG AUU CUU ACC UUA CAA A	Not tested yet
81 & 82	H20A(+44+71) & H20A(+147+168)	CUG GCA GAA UUC GAU CCA CCG GCU GUU C CAG CAG UAG UUG UCA UCU GCU C	Very strong skipping
80, 81 & 82	H19A(+35+65); H20A(+44+71); H20A(+147+168)	GCC UGA GCU GAU CUG CUG GCA UCU UGC AGU U; CUG GCA GAA UUC GAU CCA CCG GCU GUU C; CAG CAG UAG UUG UCA UCU GCU C	Very strong skipping

Antisense Oligonucleotides Directed at Exon 21

Antisense oligonucleotides directed at exon 21 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H21A(+85+108) [SEQ ID NO:92] and H21A(+85+106) [SEQ ID NO:91] induced exon 21 skipping when delivered into cells at a concentration of 50 nM. Table 16 below includes other antisense molecules tested at a concentration range of 5, 25, 50, 100, 200 and 300 nM. These antisense molecules showed a variable ability to induce exon skipping

TABLE 16

SEQ ID	Antisense Oligonucleotide name	Sequence	Ability to induce skipping
90	H21A(-06+16)	GCC GGU UGA CUU CAU CCU GUG C	Skips at 600 nM
91	H21A(+85+106)	CUG CAU CCA GGA ACA UGG GUC C	Skips at 50 nM
92	H21A(+85+108)	GUC UGC AUC CAG GAA CAU GGG UC	Skips at 50 nM
93	H21A(+08+31)	GUU GAA GAU CUG AUA GCC GGU UGA	Skips faintly to
94	H21D(+18-07)	UAC UUA CUG UCU GUA GCU CUU UCU	No skipping

Antisense Oligonucleotides Directed at Exon 22

Antisense oligonucleotides directed at exon 22 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 12 illustrates differing efficiencies of two antisense molecules directed at exon 22 acceptor splice site. H22A(+

125+106) [SEQ ID NO:96] and H22A(+80+101) [SEQ ID NO: 98] induce strong exon 22 skipping from 50 nM to 600 nM concentration.

H22A(+125+146) [SEQ ID NO:96] and H22A(+80+101) [SEQ ID NO:98] induced exon 22 skipping when delivered into cells at a concentration of 50 nM. Table 17 below shows other antisense molecules tested at a concentration range of 50, 100, 300 and 600 nM. These antisense molecules showed a variable ability to induce exon skipping.

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TABLE 17

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
95 H22A(+22+45)	CAC UCA UGG UCU CCU GAU AGC GCA	No skipping
96 H22A(+125+146)	CUG CAA UUC CCC GAG UCU CUG C	Skipping to 50 nM
97 H22A(+47+69)	ACU GCU GGA CCC AUG UCC UGA UG	Skipping to 300 nM
98 H22A(+80+101)	CUA AGU UGA GGU AUG GAG AGU	Skipping to 50 nM
99 H22D(+13-11)	UAU UCA CAG ACC UGC AAU UCC CC	No skipping

Antisense Oligonucleotides Directed at Exon 23

Antisense oligonucleotides directed at exon 23 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

Table 18 below shows antisense molecules tested at a concentration range of 25, 50, 100, 300 and 600 nM. These antisense molecules showed no ability to induce exon skipping or are yet to be tested.

TABLE 18

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
100 H23A(+34+59)	ACA GUG GUG CUG AGA UAG UAU AGG CC	No skipping
101 H23A(+18+39)	UAG GCC ACU UUG UUG CUC UUG C	No Skipping
102 H23A(+72+90)	UUC AGA GGG CGC UUU CUU C	No Skipping

Antisense Oligonucleotides Directed at Exon 24

Antisense oligonucleotides directed at exon 24 were prepared using similar methods as described above. Table 19 below outlines the antisense oligonucleotides directed at exon 24 that are yet to be tested for their ability to induce exon 24 skipping.

TABLE 19

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
103 H24A(+48+70)	GGG CAG GCC AUU CCU CCU UCA GA	Needs testing
104 H24A(-02+22)	UCU UCA GGG UUU GUA UGU GAU UCU	Needs testing

Antisense Oligonucleotides Directed at Exon 25

Antisense oligonucleotides directed at exon 25 were prepared using similar methods as described above. Table 20

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below shows the antisense oligonucleotides directed at exon 25 that are yet to be tested for their ability to induce exon 25 skipping.

TABLE 20

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
105 H25A(+9+36)	CUG GGC UGA AUU GUC UGA AUA UCA CUG	Needs testing
106 H25A(+131+156)	CUG UUG GCA CAU GUG AUC CCA CUG AG	Needs testing
107 H25D(+16-08)	GUC UAU ACC UGU UGG CAC AUG UGA	Needs testing

Antisense Oligonucleotides Directed at Exon 26

Antisense oligonucleotides directed at exon 26 were prepared using similar methods as described above. Table 21 below outlines the antisense oligonucleotides directed at exon 26 that are yet to be tested for their ability to induce exon 26 skipping.

TABLE 21

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
108 H26A(+132+156)	UGC UUU CUG UAA UUC AUC UGG AGU U	Needs testing
109 H26A(-07+19)	CCU CCU UUC UGG CAU AGA CCU UCC AC	Needs testing
110 H26A(+68+92)	UGU GUC AUC CAU UCG UGC AUC UCU G	Faint skipping at 600 nM

Antisense Oligonucleotides Directed at Exon 27

Antisense oligonucleotides directed at exon 27 were prepared using similar methods as described above. Table 22 below outlines the antisense oligonucleotides directed at exon 27 that are yet to be tested for their ability to induce exon 27 skipping.

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TABLE 22

SEQ ID name	Antisense oligonucleotide Sequence	Ability to induce skipping
111 H27A(+82+106)	UUA AGG CCU CUU GUG CUA CAG GUG G	Needs testing
112 H27A(-4+19)	GGG CCU CUU CUU UAG CUC UCU GA	Faint skipping at 600 and 300 nM
113 H27D(+19-03)	GAC UUC CAA AGU CUU GCA UUU C	v. strong skipping at 600 and 300 nM

Antisense Oligonucleotides Directed at Exon 28

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Antisense oligonucleotides directed at exon 28 were prepared using similar methods as described above. Table 23 below outlines the antisense oligonucleotides directed at exon 28 that are yet to be tested for their ability to induce exon 28 skipping.

TABLE 23

SEQ ID name	Antisense oligonucleotide Sequence	Ability to induce skipping
114 H28A(-05+19)	GCC AAC AUG CCC AAA CUU CCU AAG	v. strong skipping at 600 and 300 nM
115 H28A(+99+124)	CAG AGA UUU CCU CAG CUC CGC CAG GA	Needs testing
116 H28D(+16-05)	CUU ACA UCU AGC ACC UCA GAG	v. strong skipping at 600 and 300 nM

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Antisense Oligonucleotides Directed at Exon 29

Antisense oligonucleotides directed at exon 29 were prepared using similar methods as described above. Table 24 below outlines the antisense oligonucleotides directed at exon 29 that are yet to be tested for their ability to induce exon 29 skipping.

TABLE 24

SEQ ID name	Antisense oligonucleotide Sequence	Ability to induce skipping
117 H29A(+57+81)	UCC GCC AUC UGU UAG GGU CUG UGC C	Needs testing
118 H29A(+18+42)	AUU UGG GUU AUC CUC UGA AUG UCG C	v. strong skipping at 600 and 300 nM
119 H29D(+17-05)	CAU ACC UCU UCA UGU AGU UCC C	v. strong skipping at 600 and 300 nM

Antisense Oligonucleotides Directed at Exon 30

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Antisense oligonucleotides directed at exon 30 were prepared using similar methods as described above. Table 25 below outlines the antisense oligonucleotides directed at exon 30 that are yet to be tested for their ability to induce exon 30 skipping.

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TABLE 25

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
120 H30A (+122+147)		CAU UUG AGC UGC GUC CAC CUU GUC UG	Needs testing
121 H30A (+25+50)		UCC UGG GCA GAC UGG AUG CUC UGU UC	Very strong skipping at 600 and 300 nM.
122 H30D (+19-04)		UUG CCU GGG CUU CCU GAG GCA UU	Very strong skipping at 600 and 300 nM.

Antisense Oligonucleotides Directed at Exon 31

Antisense oligonucleotides directed at exon 31 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 13 illustrates differing efficiencies of two antisense molecules directed at exon 31 acceptor splice site and a

15 "cocktail" of exon 31 antisense oligonucleotides at varying concentrations. H31D(+03-22) [SEQ ID NO:124] substantially induced exon 31 skipping when delivered into cells at a concentration of 20 nM. Table 26 below also includes other antisense molecules tested at a concentration of 100 and 300 nM. These antisense molecules showed a variable ability to induce exon skipping.

TABLE 26

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
123 H31D (+06-18)		UUC UGA AAU AAC AUA UAC CUG UGC	Skipping to 300 nM
124 H31D (+03-22)		UAG UUU CUG AAA UAA CAU AUA CCU G	Skipping to 20 nM
125 H31A (+05+25)		GAC UUG UCA AAU CAG AUU GGA	No skipping
126 H31D (+04-20)		GUU UCU GAA AUA ACA UAU ACC UGU	Skipping to 300 nM

Antisense Oligonucleotides Directed at Exon 32

40 Antisense oligonucleotides directed at exon 32 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H32D(+04-16) [SEQ ID NO:127] and H32A(+49+73) 45 [SEQ ID NO:130] induced exon 32 skipping when delivered into cells at a concentration of 300 nM. Table 27 below also shows other antisense molecules tested at a concentration of 100 and 300 nM. These antisense molecules did not show an ability to induce exon skipping.

TABLE 27

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
127H32D (+04-16)		CAC CAG AAA UAC AUA CCA CA	Skipping to 300 nM
128H32A (+151+170)		CAA UGA UUU AGC UGU GAC UG	No skipping
129H32A (+10+32)		CGA AAC UUC AUG GAG ACA UCU UG	No skipping
130H32A (+49+73)		CUU GUA GAC GCU GCU CAA AAU UGG C	Skipping to 300 nM

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Antisense Oligonucleotides Directed at Exon 33

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Antisense oligonucleotides directed at exon 33 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 14 shows differing efficiencies of two antisense molecules directed at exon 33 acceptor splice site. H33A(+64+88) [SEQ ID NO:134] substantially induced exon 33 skipping when delivered into cells at a concentration of 10 nM. Table 28 below includes other antisense molecules tested at a concentration of 100, 200 and 300 nM. These antisense molecules showed a variable ability to induce exon skipping.

TABLE 28

SEQ ID name	Antisense oligonucleotide Sequence	Ability to induce skipping
131 H33D (+09-11)	CAU GCA CAC ACC UUU GCU CC	No skipping
132 H33A (+53+76)	UCU GUA CAA UCU GAC GUC CAG UCU	Skipping to 200 nM
133 H33A (+30+56)	GUG UUU AUC ACC AUU UCC ACU UCA GAC	Skipping to 200 nM
134 H33A (+64+88)	GCG UCU GCU UUU UCU GUA CAA UCU G	Skipping to 10 nM

Antisense Oligonucleotides Directed at Exon 34

Antisense oligonucleotides directed at exon 34 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

Table 29 below includes antisense molecules tested at a concentration of 100 and 300 nM. These antisense molecules showed a variable ability to induce exon skipping.

TABLE 29

SEQ ID name	Antisense oligonucleotide Sequence	Ability to induce skipping
135 H34A (+83+104)	UCC AUA UCU GUA GCU GGC AGC C	No skipping
136 H34A (+143+165)	CCA GGC AAC UUC AGA AUC CAA AU	No skipping
137 H34A (-20+10)	UUU CUG UUA CCU GAA AAG AAU UAU AAU GAA	Not tested
138 H34A (+46+70)	CAU UCA UUU CCU UUC GCA UCU UAC G	Skipping to 300 nM
139 H34A (+95+120)	UGA UCU CUU UGU CAA UUC CAU AUC UG	Skipping to 300 nM
140 H34D (+10-20)	UUC AGU GAU AUA GGU UUU ACC UUU CCC CAG	Not tested
141 H34A (+72+96)	CUG UAG CUG CCA GCC AUU CUG UCA AG	No skipping

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Antisense Oligonucleotides Directed at Exon 35

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Antisense oligonucleotides directed at exon 35 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above. 5

FIG. 15 shows differing efficiencies of antisense molecules directed at exon 35 acceptor splice site. H35A(+24+43) [SEQ ID NO:144] substantially induced exon 35 skipping when delivered into cells at a concentration of 20 nM. Table 30 below also includes other antisense molecules 10 tested at a concentration of 100 and 300 nM. These antisense molecules showed no ability to induce exon skipping.

TABLE 30

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
142	H35A (+141+161)	UCU UCU GCU CGG GAG GUG ACA	Skipping to 20 nM
143	H35A (+116+135)	CCA GUU ACU AUU CAG AAG AC	No skipping
144	H35A (+24+43)	UCU UCA GGU GCA CCU UCU GU	No skipping

Antisense Oligonucleotides Directed at Exon 36 25

Antisense oligonucleotides directed at exon 36 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

Antisense molecule H36A(+26+50) [SEQ ID NO:145] 30 induced exon 36 skipping when delivered into cells at a concentration of 300 nM, as shown in FIG. 16.

Antisense Oligonucleotides Directed at Exon 37 35

Antisense oligonucleotides directed at exon 37 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 17 shows differing efficiencies of two antisense 40 molecules directed at exon 37 acceptor splice site. H37A(+82+105) [SEQ ID NO:148] and H37A(+134+157) [SEQ ID NO:149] substantially induced exon 37 skipping when delivered into cells at a concentration of 10 nM. Table 31 below shows the antisense molecules tested.

TABLE 31

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
147	H37A (+26+50)	CGU GUA GAG UCC ACC UUU GGG CGU A	No skipping
148	H37A (+82+105)	UAC UAA UUU CCU GCA GUG GUC ACC	Skipping to 10 nM
149	H37A (+134+157)	UUC UGU GUG AAA UGG CUG CAA AUC	Skipping to 10 nM

Antisense Oligonucleotides Directed at Exon 38

Antisense oligonucleotides directed at exon 38 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above. 60

FIG. 18 illustrates antisense molecule H38A(+88+112) [SEQ ID NO:152], directed at exon 38 acceptor splice site. H38A(+88+112) substantially induced exon 38 skipping 65 when delivered into cells at a concentration of 10 nM. Table 32 below shows the antisense molecules tested and their ability to induce exon skipping.

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TABLE 32

Antisense SEQ ID name	Sequence	Ability to induce skipping
150H38A (-01+19)	CCU UCA AAG GAA UGG AGG CC	No skipping
151H38A (+59+83)	UGC UGA AUU UCA GCC UCC AGU GGU U	Skipping to 10 nM
152H38A (+88+112)	UGA AGU CUU CCU CUU UCA GAU UCA C	Skipping to 10 nM

Antisense Oligonucleotides Directed at Exon 39

Antisense oligonucleotides directed at exon 39 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H39A(+62+85) [SEQ ID NO:153] induced exon 39 skipping when delivered into cells at a concentration of 100 nM. Table 33 below shows the antisense molecules tested and their ability to induce exon skipping.

TABLE 33

Antisense SEQ ID name	Sequence	Ability to induce skipping
153 H39A (+62+85)	CUG GCU UUC UCU CAU CUG UGA UUC	Skipping to 100 nM
154 H39A (+39+58)	GUU GUA AGU UGU CUC CUC UU	No skipping
155 H39A (+102+121)	UUG UCU GUA ACA GCU GCU GU	No skipping
156 H39D (+10-10)	GCU CUA AUA CCU UGA GAG CA	Skipping to 300 nM

Antisense Oligonucleotides Directed at Exon 40

Antisense oligonucleotides directed at exon 40 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 19 illustrates antisense molecule H40A(-05+17) [SEQ ID NO:157] directed at exon 40 acceptor splice site. H40A(-05+17) and H40A(+129+153) [SEQ ID NO:158] both substantially induced exon 40 skipping when delivered into cells at a concentration of 5 nM.

Antisense Oligonucleotides Directed at Exon 42

Antisense oligonucleotides directed at exon 42 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 20 illustrates antisense molecule H42A(-04+23) [SEQ ID NO:159], directed at exon 42 acceptor splice site. H42A(-4+23) and H42D(+19-02) [SEQ ID NO:161] both induced exon 42 skipping when delivered into cells at a concentration of 5 nM. Table 34 below shows the antisense molecules tested and their ability to induce exon 42 skipping.

TABLE 34

Antisense afigonucleotide SEQ ID name	Sequence	Ability to induce skipping
159 H42A (-4+23)	AUC GUU UCU UCA CGG ACA GUG UGG UGC	Skipping to 5 nM
160 H42A (+86+109)	GGG CUU GUG AGA CAU GAG UGA UUU	Skipping to 100 nM
161 H42D (+19-02)	A CCU UCA GAG GAC UCC UCU UGC	Skipping to 5 nM

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Antisense Oligonucleotides Directed at Exon 43

Antisense oligonucleotides directed at exon 43 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H43A(+101+120) [SEQ ID NO:163] induced exon 43 skipping when delivered into cells at a concentration of 25 nM. Table 35 below includes the antisense molecules tested and their ability to induce exon 43 skipping.

TABLE 35

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
162	H43D (+10-15)	UAU GUG UUA CCU ACC CUU GUC GGU C	Skipping to 100 nM
163	H43A (+101+120)	GGA GAG AGC UUC CUG UAG CU	Skipping to 25 nM
164	H43A (+78+100)	UCA CCC UUU CCA CAG GCG UUG CA	Skipping to 200 nM

Antisense Oligonucleotides Directed at Exon 44

Antisense oligonucleotides directed at exon 44 were prepared using similar methods as described above. Testing for the ability of these antisense molecules to induce exon 44 skipping is still in progress. The antisense molecules under review are shown as SEQ ID Nos: 165 to 167 in Table 1A.

Antisense Oligonucleotides Directed at Exon 45

Antisense oligonucleotides directed at exon 45 were prepared using similar methods as described above. Testing for the ability of these antisense molecules to induce exon 45 skipping is still in progress. The antisense molecules under review are shown as SEQ ID Nos: 207 to 211 in Table 1A.

Antisense Oligonucleotides Directed at Exon 46

Antisense oligonucleotides directed at exon 46 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 21 illustrates the efficiency of one antisense molecule directed at exon 46 acceptor splice site. Antisense oligonucleotide H46A(+86+115) [SEQ ID NO:203] showed very strong ability to induce exon 46 skipping. Table 36 below includes antisense molecules tested. These antisense molecules showed varying ability to induce exon 46 skipping.

TABLE 36

SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
168	H46D (+16-04)	UUA CCU UGA CUU GCU CAA GC	No skipping
169	H46A (+90+109)	UCC AGG UUC AAG UGG GAU AC	No skipping
203	H46A (+86+115)	CUC UUU UCC AGG UUC AAG UGG GAU ACU AGC	Good skipping to 100 nM
204	H46A (+107+137)	CAA GCU UUU CUU UUA GUU GCU GCU CUU UUC C	Good skipping to 100 nM
205	H46A (-10+20)	UAU UCU UUU GUU CUU CUA GCC UGG AGA AAG	Weak skipping
206	H46A (+50+77)	CUG CUU CCU CCA ACC AUA AAA CAA AUU C	Weak skipping

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Antisense Oligonucleotides Directed at Exon 47

Antisense oligonucleotides directed at exon 47 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

H47A(+76+100) [SEQ ID NO:170] and H47A(-09+12) [SEQ ID NO:172] both induced exon 47 skipping when delivered into cells at a concentration of 200 nM. H47D(+25-02) [SEQ ID NO: 171] is yet to be prepared and tested.

Antisense Oligonucleotides Directed at Exon 50

Antisense oligonucleotides directed at exon 50 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

Antisense oligonucleotide molecule H50A(+02+30) [SEQ ID NO: 173] was a strong inducer of exon skipping. Further, H50A(+07+33) [SEQ ID NO:174] and H50D(+07-18) [SEQ ID NO:175] both induced exon 50 skipping when delivered into cells at a concentration of 100 nM.

Antisense Oligonucleotides Directed at Exon 51

Antisense oligonucleotides directed at exon 51 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 22 illustrates differing efficiencies of two antisense molecules directed at exon 51 acceptor splice site. Antisense oligonucleotide H51A(+66+90) [SEQ ID NO:180] showed the stronger ability to induce exon 51 skipping. Table 37 below includes antisense molecules tested at a concentration range of 25, 50, 100, 300 and 600 nM. These antisense molecules showed varying ability to induce exon 51 skipping. The strongest inducers of exon skipping were antisense oligonucleotide H51A(+61+90) [SEQ ID NO: 179] and H51A(+66+95) [SEQ ID NO: 181].

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TABLE 37

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
176 H51A (-01+25)	ACC AGA GUA ACA GUC UGA GUA GGA GC	Faint skipping
177 H51D (+16-07)	CUC AUA CCU UCU GCU UGA UGA UC	Skipping at 300 nM
178 H51A (+111+134)	UUC UGU CCA AGC CCG GUU GAA AUC	Needs re-testing
179 H51A (+61+90)	ACA UCA AGG AAG AUG GCA UUU CUA GUU UGG	Very strong skipping
180 H51A (+66+90)	ACA UCA AGG AAG AUG GCA UUU CUA G	skipping
181 H51A (+66+95)	CUC CAA CAU CAA GGA AGA UGG CAU UUC UAG	Very strong skipping
182 H51D (+08-17)	AUC AUU UUU UCU CAU ACC UUC UGC U	No skipping
183 H51A/D (+08-17) & (-15+?)	AUC AUU UUU UCU CAU ACC UUC UGC UAG GAG CUA AAA	No skipping
184 H51A (+175+195)	CAC CCA CCA UCA GCC UCU GUG	No skipping
185 H51A (+199+220)	AUC AUC UCG UUG AUA UCC UCA A	No skipping

Antisense Oligonucleotides Directed at Exon 52

Antisense oligonucleotides directed at exon 52 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 22 also shows differing efficiencies of four antisense molecules directed at exon 52 acceptor splice site. The most effective antisense oligonucleotide for inducing exon 52 skipping was H52A(+17+37) [SEQ ID NO:188].

Table 38 below shows antisense molecules tested at a concentration range of 50, 100, 300 and 600 nM. These antisense molecules showed varying ability to induce exon 50 skipping. Antisense molecules H52A(+12+41) [SEQ ID NO:187] and H52A(+17+37) [SEQ ID NO:188] showed the strongest exon 50 skipping at a concentration of 50 nM.

TABLE 38

Antisense oligonucleotide SEQ ID name	Sequence	Ability to induce skipping
186H52A (-07+14)	UCC UGC AUU GUU GCC UGU AAG	No skipping
187H52A (+12+41)	UCC AAC UGG GGA CGC CUC UGU AAA UCC	Very strong skipping
188H52A (+17+37)	ACU GGG GAC GCC UCU GUU CCA	Skipping to 50 nM
189H52A (+93+112)	CCG UAA UGA UUG UUC UAG CC	No skipping
190H52D (+05-15)	UGU UAA AAA ACU UAC UUC GA	No skipping

Antisense Oligonucleotides Directed at Exon 53

Antisense oligonucleotides directed at exon 53 were prepared and tested for their ability to induce exon skipping in human muscle cells using similar methods as described above.

FIG. 22 also shows antisense molecule H53A(+39+69) [SEQ ID NO:193] directed at exon 53 acceptor splice site. This antisense oligonucleotide was able to induce exon 53 skipping at 5, 100, 300 and 600 nM. A "cocktail" of three exon 53 antisense oligonucleotides: H53A(+23+47) [SEQ ID NO:195], H53A(+150+176) [SEQ ID NO:196] and H53D(+14-07) [SEQ ID NO:194], was also tested, as shown in FIG. 20 and exhibited an ability to induce exon skipping.

Table 39 below includes other antisense molecules tested at a concentration range of 50, 100, 300 and 600 nM. These antisense molecules showed varying ability to induce exon 53 skipping. Antisense molecule H53A(+39+69) [SEQ ID NO:193] induced the strongest exon 53 skipping.

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TABLE 39

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SEQ ID name	Antisense oligonucleotide	Sequence	Ability to induce skipping
191 H53A (+45+69)		CAU UCA ACU GUU GCC UCC GGU UCU G	Faint skipping at 50 nM
192 H53A (+39+62)		CUG UUG CCU CCG GUU CUG AAG GUG	Faint skipping at 50 nM
193 H53A (+39+69)		CAU UCA ACU GUU GCC UCC GGU UCU GAA GGU G	Strong skipping to 50 nM
194 H53D (+14-07)		UAC UAA CCU UGG UUU CUG UGA	Very faint skipping to 50 nM
195 H53A (+23+47)		CUG AAG GUG UUC UUG UAC UUC AUC C	Very faint skipping to 50 nM
196 H53A (+150+176)		UGU AUA GGG ACC CUC CUU CCA UGA CUC	Very faint skipping to 50 nM
197 H53D (+20-05)		CUA ACC UUG GUU UCU GUG AUU UUC U	Not made yet
198 H53D (+09-18)		GGU AUC UUU GAU ACU AAC CUU GGU UUC	Faint at 600 nM
199 H53A (-12+10)		AUU CUU UCA ACU AGA AUA AAA G	No skipping
200 H53A (-07+18)		GAU UCU GAA UUG UUU CAA CUA GAA U	No skipping
201 H53A (+07+26)		AUC CCA CUG AUU CUG AAU UC	No skipping
202 H53A (+124+145)		UUG GCU CUG GCC UGU CCU AAG A	No skipping

SEQUENCE LISTING

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<211> LENGTH: 24

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

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24

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

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gauagguggu aucaacaucu g

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<212> TYPE: RNA

67

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 oligonucleotide

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 oligonucleotide

<400> SEQUENCE: 5

guaucaacu cuuagagac 20

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 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 7

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auuuaccaac cuuaggaua gagua 25

69

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<400> SEQUENCE: 10

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<400> SEQUENCE: 11

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<400> SEQUENCE: 12

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oligonucleotide

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guggucuccu uaccuaugac ugugg

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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ugucucagua aucuucuuac cuau

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<212> TYPE: RNA

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<212> TYPE: RNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 19

gcaugaacuc uuguggaucc

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<210> SEQ ID NO 20

<211> LENGTH: 20

73

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<212> TYPE: RNA
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Human 2'-O-methyl phosphorothioate antisense
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<400> SEQUENCE: 20

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<400> SEQUENCE: 21

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<210> SEQ ID NO 22
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<212> TYPE: RNA
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<400> SEQUENCE: 24

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Human 2'-O-methyl phosphorothioate antisense
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<400> SEQUENCE: 25

75

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gcgcuccca uccuguaggu cacug

25

<210> SEQ ID NO 26
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oligonucleotide

<400> SEQUENCE: 26

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 27

cucccauccu guaggucacu g

21

<210> SEQ ID NO 28
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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oligonucleotide

<400> SEQUENCE: 28

uaccaguuuu ugcccuguca gg

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 29

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<210> SEQ ID NO 30
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 30

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<210> SEQ ID NO 31
<211> LENGTH: 31
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 31

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 33

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<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 34

cgaugucagu acuccaaua uucac

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<210> SEQ ID NO 35

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<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 35

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<210> SEQ ID NO 36

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<212> TYPE: RNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 36

accugccagu ggaggauu

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<210> SEQ ID NO 37

79

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oligonucleotide

<400> SEQUENCE: 37

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oligonucleotide

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

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31

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<212> TYPE: RNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

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oligonucleotide

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oligonucleotide

<400> SEQUENCE: 42

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oligonucleotide

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oligonucleotide

<400> SEQUENCE: 44

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oligonucleotide

<400> SEQUENCE: 45

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<210> SEQ ID NO 46
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oligonucleotide

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<212> TYPE: RNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

<400> SEQUENCE: 47

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<210> SEQ ID NO 48
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<220> FEATURE:

83

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oligonucleotide

<400> SEQUENCE: 48

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

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caucuucuga uaaauuuccu guu

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<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

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<210> SEQ ID NO 51

<211> LENGTH: 20

<212> TYPE: RNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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oligonucleotide

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85

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oligonucleotide

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oligonucleotide

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oligonucleotide

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Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

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caucuacaga uguuugccca uc

22

<210> SEQ ID NO 58
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 58

gaagggauguc uuguaaaaga acc

23

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

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<400> SEQUENCE: 59

accuguucuu caguaagacg

20

<210> SEQ ID NO 60

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 60

caugacacac cuguucuca guaa

24

<210> SEQ ID NO 61

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 61

cauuugagaa ggaugucuug

20

<210> SEQ ID NO 62

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 62

aucucccaau accuggagaa gaga

24

<210> SEQ ID NO 63

<211> LENGTH: 31

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 63

gccaugcacu aaaaaggcac ugcaagacau u

31

<210> SEQ ID NO 64

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 64

ucuuuaaagc caguugugug aauc

24

<210> SEQ ID NO 65

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 65

uuucugaaag ccaugcacua a

21

<210> SEQ ID NO 66
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 66

guacauacgg ccaguuuuug aagac

25

<210> SEQ ID NO 67
<211> LENGTH: 31
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 67

cuagaucgcg uuuuuaaaacc uguuuaaaca a

31

<210> SEQ ID NO 68
<211> LENGTH: 31
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 68

ucuuuuucag auccgcuuuu aaaaccuguu a

31

<210> SEQ ID NO 69
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 69

cuagaucgcg uuuuuaaaacc uguua

25

<210> SEQ ID NO 70
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 70

ccgucuuucg ggucacugac uua

23

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<210> SEQ ID NO 71
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 71

cuagaucgcg uuuuaaaacc uguuaa

26

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 72

ccgcuuuuuaa aaccuguuuaa

20

<210> SEQ ID NO 73
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 73

uggauugcuu uuucuuuucu agaucc

26

<210> SEQ ID NO 74
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 74

caugcuuccg ucuucugggu cacug

25

<210> SEQ ID NO 75
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 75

gaucuuguuu gagugaauc agu

23

<210> SEQ ID NO 76
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

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<400> SEQUENCE: 76

guuauccagc caugcuuccg uc

22

<210> SEQ ID NO 77

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 77

ugauaaauugg uaucacuaac cugug

25

<210> SEQ ID NO 78

<211> LENGTH: 22

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 78

guaucaacuaa ccugugcugu ac

22

<210> SEQ ID NO 79

<211> LENGTH: 19

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 79

cugcuggcgu cuugcaguu

19

<210> SEQ ID NO 80

<211> LENGTH: 31

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 80

gccugagcug aucugcuggc aucuugcagu u

31

<210> SEQ ID NO 81

<211> LENGTH: 28

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 81

cuggcagaau ucgauccacc ggcuguuc

28

<210> SEQ ID NO 82

<211> LENGTH: 22

<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 82

cagcaguagu ugucaucugc uc

22

<210> SEQ ID NO 83

<211> LENGTH: 19

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 83

ugauggggug guggguugg

19

<210> SEQ ID NO 84

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 84

aucugcauua acacccucua gaaag

25

<210> SEQ ID NO 85

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 85

ccggcuguuc aguuguucug aggc

24

<210> SEQ ID NO 86

<211> LENGTH: 28

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 86

aucugcauua acacccucua gaaagaaa

28

<210> SEQ ID NO 87

<211> LENGTH: 28

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 87

gaaggagaag agauucuua cuuacaaa

28

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<210> SEQ ID NO 88
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 88

auucgaucca ccggcuguuc

20

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 89

cagcaguagu ugucaucugc

20

<210> SEQ ID NO 90
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 90

gccggguagac uucauccugu gc

22

<210> SEQ ID NO 91
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 91

cugcauccag gaacaugggu cc

22

<210> SEQ ID NO 92
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 92

gucugcaucc aggaacaugg guc

23

<210> SEQ ID NO 93
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense

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oligonucleotide

<400> SEQUENCE: 93

guugaagauc ugauagccgg uuga

24

<210> SEQ ID NO 94

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 94

uacuuacugu cuguagcucu uucu

24

<210> SEQ ID NO 95

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 95

cacucauggu cuccugauag cgca

24

<210> SEQ ID NO 96

<211> LENGTH: 22

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 96

cugcaauucc ccgagucucu gc

22

<210> SEQ ID NO 97

<211> LENGTH: 23

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 97

acugcuggac ccauguccug aug

23

<210> SEQ ID NO 98

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 98

quaaguagag guauggagag u

21

<210> SEQ ID NO 99

<211> LENGTH: 23

101

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 99
uauucacaga ccugcaauuc ccc
23

<210> SEQ ID NO 100
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 100
acaguggugc ugagauagua uaggcc
26

<210> SEQ ID NO 101
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 101
uagggcacuu uguugcucuu gc
22

<210> SEQ ID NO 102
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 102
uucagagggc gcuuucuuuc
19

<210> SEQ ID NO 103
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 103
gggcaggcca uuccuccuuc aga
23

<210> SEQ ID NO 104
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 104

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uccuucagggg uuguauuguga uucu

24

<210> SEQ ID NO 105
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 105

cugggcugaa uugucugaau aucacug

27

<210> SEQ ID NO 106
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 106

cuguuggcac augugaucac acugag

26

<210> SEQ ID NO 107
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 107

gucuaauaccu guuggcacau guga

24

<210> SEQ ID NO 108
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 108

ugcuuucugu aaaucaucug gaguu

25

<210> SEQ ID NO 109
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 109

ccuccuuucu ggcauagacc uuccac

26

<210> SEQ ID NO 110
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 110

ugugucaucc auucgugcau cucug

25

<210> SEQ ID NO 111

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 111

uuaagggcuc uugugcuaca ggugg

25

<210> SEQ ID NO 112

<211> LENGTH: 23

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 112

gggcucucuu uuagcucuc uga

23

<210> SEQ ID NO 113

<211> LENGTH: 22

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 113

gacuuccaaa gucuugcauu uc

22

<210> SEQ ID NO 114

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 114

gccaacaugc ccaaacuucc uaag

24

<210> SEQ ID NO 115

<211> LENGTH: 26

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 115

cagagauuuc cucagcuccg ccaggga

26

<210> SEQ ID NO 116

107

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<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 116

cuuacauca gacaccucaga g

21

<210> SEQ ID NO 117
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 117

uccgccaucu guuagggucu gugcc

25

<210> SEQ ID NO 118
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 118

auuuggguaa uccucugaau guccg

25

<210> SEQ ID NO 119
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 119

cauaccucuu cauguaguuc cc

22

<210> SEQ ID NO 120
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 120

cauuugagcu gcuuccaccu ugucug

26

<210> SEQ ID NO 121
<211> LENGTH: 26
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 121

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uccugggcag acuggaugcu cuguuc

26

<210> SEQ ID NO 122
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 122

uugccugggc uuccugagggc auu

23

<210> SEQ ID NO 123
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 123

uucugaaaua acauauaccu gugc

24

<210> SEQ ID NO 124
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 124

uaguucuga aauaacauau accug

25

<210> SEQ ID NO 125
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 125

gacuugucaa aucagauugg a

21

<210> SEQ ID NO 126
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 126

guuucugaaa uaacauauac cugu

24

<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 127

caccagaaaU acauaccaca

20

<210> SEQ ID NO 128

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 128

caaugauuuU gcugugacug

20

<210> SEQ ID NO 129

<211> LENGTH: 23

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 129

cgaaacuuUa uggagacauc uug

23

<210> SEQ ID NO 130

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 130

cuuguagacg cugcucaaaa uuggc

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<210> SEQ ID NO 131

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 131

caugcacaca ccuuugcuUc

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<210> SEQ ID NO 132

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 132

ucuguacaau cugacgucca gucu

24

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<210> SEQ ID NO 133
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide
<400> SEQUENCE: 133

gucuuuauca ccauuuccac uucagac

27

<210> SEQ ID NO 134
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide
<400> SEQUENCE: 134

ccgucugcuu uuucuguaca aucug

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<210> SEQ ID NO 135
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide
<400> SEQUENCE: 135

uccauaucug uagcugccag cc

22

<210> SEQ ID NO 136
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide
<400> SEQUENCE: 136

ccaggcaacu ucagaaucua auu

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<210> SEQ ID NO 137
<211> LENGTH: 30
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide
<400> SEQUENCE: 137

uuucuguuac cugaaaagaa uuauaaugaa

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<210> SEQ ID NO 138
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

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<400> SEQUENCE: 138

cauucuuuc cuuucgcauc uuacg

25

<210> SEQ ID NO 139

<211> LENGTH: 26

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 139

ugaucucuuu gucaauucca uaucug

26

<210> SEQ ID NO 140

<211> LENGTH: 30

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 140

uucagugaua uagguuuuac cuuuccccag

30

<210> SEQ ID NO 141

<211> LENGTH: 26

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 141

cuguagcugc cagccauucg gucaag

26

<210> SEQ ID NO 142

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 142

ucuucugcuc gggaggugac a

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<210> SEQ ID NO 143

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 143

ccaguuacua uucagaagac

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<210> SEQ ID NO 144

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 144

uouucaggug cacuucugu

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<210> SEQ ID NO 145
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 145

ugugaugugg uccacauucu gguca

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<210> SEQ ID NO 146
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 146

ccauguguuu cuggauuucc

20

<210> SEQ ID NO 147
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 147

cguguagagu ccaccuuugg gcgua

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<210> SEQ ID NO 148
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 148

uacuaauuuc cugcaguggu cacc

24

<210> SEQ ID NO 149
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 149

uucuguguga aauggcugca aauc

24

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120

<210> SEQ ID NO 150
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 150

ccucaaagg auggaggcc

20

<210> SEQ ID NO 151
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 151

ugcugaauu cagccuccag ugguu

25

<210> SEQ ID NO 152
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 152

ugaagucuc cucuucaga uucac

25

<210> SEQ ID NO 153
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 153

cuggcuuuc cucaucugug auuc

24

<210> SEQ ID NO 154
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 154

guuguaaguu gucuccucu

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<210> SEQ ID NO 155
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

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<400> SEQUENCE: 155

uugucguuaa cagcugcugu

20

<210> SEQ ID NO 156

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 156

gcucuaauac cuugagagca

20

<210> SEQ ID NO 157

<211> LENGTH: 22

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 157

cuuugagacc ucaaauccug uu

22

<210> SEQ ID NO 158

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 158

cuuuauuuuc cuuucaucuc ugggc

25

<210> SEQ ID NO 159

<211> LENGTH: 27

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 159

aucguuucuu cacggacagu gugcugg

27

<210> SEQ ID NO 160

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 160

Bggcuuguga gacaugagug auuu

24

<210> SEQ ID NO 161

<211> LENGTH: 22

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      Human 2'-O-methyl phosphorothioate antisense
      oligonucleotide

<400> SEQUENCE: 161

accuucagag gacuccucuu gc
22

<210> SEQ ID NO 162
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      Human 2'-O-methyl phosphorothioate antisense
      oligonucleotide

<400> SEQUENCE: 162

uauuguuuac cuacccuugu cgguc
25

<210> SEQ ID NO 163
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      Human 2'-O-methyl phosphorothioate antisense
      oligonucleotide

<400> SEQUENCE: 163

ggagagagcu uccuguagcu
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<210> SEQ ID NO 164
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      Human 2'-O-methyl phosphorothioate antisense
      oligonucleotide

<400> SEQUENCE: 164

ucacccuuuc cacaggcgau gca
23

<210> SEQ ID NO 165
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      Human 2'-O-methyl phosphorothioate antisense
      oligonucleotide

<400> SEQUENCE: 165

uuugugucuu ucugagaaac
20

<210> SEQ ID NO 166
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      Human 2'-O-methyl phosphorothioate antisense
      oligonucleotide

<400> SEQUENCE: 166

aaagacuuac cuuaagauac
20

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<210> SEQ ID NO 167
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 167

aucugucaaa ucgccugcag

20

<210> SEQ ID NO 168
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 168

guaccuugac uugcucaagc

20

<210> SEQ ID NO 169
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 169

uccagguuca agugggauac

20

<210> SEQ ID NO 170
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 170

gcucucucgg gcuaauggga gcacu

25

<210> SEQ ID NO 171
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 171

accuuuaucc acuggagauu ugucugc

27

<210> SEQ ID NO 172
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

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<400> SEQUENCE: 172

uuccaccagu aacugaaaca g

21

<210> SEQ ID NO 173

<211> LENGTH: 29

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 173

ccacucagag cucagaucuu cuaacuucc

29

<210> SEQ ID NO 174

<211> LENGTH: 27

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 174

cuuccacuca gagcucagau cuucuaa

27

<210> SEQ ID NO 175

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 175

gggauccagu auacuuacag gcucc

25

<210> SEQ ID NO 176

<211> LENGTH: 26

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 176

accagaguaa cagucugagu aggagc

26

<210> SEQ ID NO 177

<211> LENGTH: 23

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Human 2'-O-methyl phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 177

cucuuaccuu cugcuugaug auc

23

<210> SEQ ID NO 178

<211> LENGTH: 24

<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 178

uucuguccaa gcccgguuga aauc

24

<210> SEQ ID NO 179

<211> LENGTH: 30

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 179

acaucaagga agauggcauu ucuaguugg

30

<210> SEQ ID NO 180

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 180

acaucaagga agauggcauu ucuag

25

<210> SEQ ID NO 181

<211> LENGTH: 30

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 181

uccaacauc aaggaagaug gcuuuucua

30

<210> SEQ ID NO 182

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 182

aucauuuuuu cucuuaccuu cugcu

25

<210> SEQ ID NO 183

<211> LENGTH: 36

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 183

aucauuuuuu cucuuaccuu cugcuaggag cuaaaa

36

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<210> SEQ ID NO 184
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 184

caccacacau caccucugu g

21

<210> SEQ ID NO 185
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 185

aucaucucgu ugauaucuc aa

22

<210> SEQ ID NO 186
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 186

uccugcaug uugccuguaa g

21

<210> SEQ ID NO 187
<211> LENGTH: 30
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 187

accaacuggg gagccucug uuccaaaucc

30

<210> SEQ ID NO 188
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 188

acuggggacy ccucuguucc a

21

<210> SEQ ID NO 189
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense

133

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oligonucleotide

<400> SEQUENCE: 189

cguuaugau uguuagcc

20

<210> SEQ ID NO 190

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 190

uguaaaaaa cuuacuucg

20

<210> SEQ ID NO 191

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 191

cauuaacug uugccuccg uucug

25

<210> SEQ ID NO 192

<211> LENGTH: 24

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 192

cuguugcuc cgguucugaa ggug

24

<210> SEQ ID NO 193

<211> LENGTH: 31

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 193

cauuaacug uugccuccg uucugaaggu g

31

<210> SEQ ID NO 194

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 194

uacuaaccuu gguuucugug a

21

<210> SEQ ID NO 195

<211> LENGTH: 25

135

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 195

cugaaggugu ucuuguacuu caucc

25

<210> SEQ ID NO 196
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 196

uguauaggga cccuccuucc augacuc

27

<210> SEQ ID NO 197
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 197

uaaaccuugg uuucugugau uuucu

25

<210> SEQ ID NO 198
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 198

gguaucuuug auacuaaccu ugguuuc

27

<210> SEQ ID NO 199
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 199

auucuuucaa cuagaauaaa ag

22

<210> SEQ ID NO 200
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
Human 2'-O-methyl phosphorothioate antisense
oligonucleotide

<400> SEQUENCE: 200

gauucugaau ucuuuaacu agaau

25

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139

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140

-continued

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US 9,994,851 B2

142

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What is claimed is:

1. An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

2. A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base

sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,994,851 B2
APPLICATION NO. : 15/705172
DATED : June 12, 2018
INVENTOR(S) : Wilton et al.

Page 1 of 1

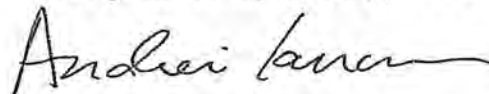
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1, Line 26, before "STATEMENT REGARDING SEQUENCE LISTING", insert:
--STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant number R01 NS044146 awarded by the National Institutes of Health. The government has certain rights in the invention.--

Signed and Sealed this
Thirty-first Day of July, 2018



Andrei Iancu
Director of the United States Patent and Trademark Office

Exhibit 10

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: August 11, 2014
Electronic Signature for Amy E. Mandragouras, Esq.: /Amy E. Mandragouras, Esq./

Docket No.: AVN-008CN26
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Stephen Donald Wilton *et al.*

Application No.: 14/316,609

Confirmation No.: 4848

Filed: June 26, 2014

Art Unit: Not Yet Assigned

For: ANTISENSE OLIGONUCLEOTIDES FOR
INDUCING EXON SKIPPING
AND METHODS OF USE THEREOF

Examiner: Not Yet Assigned

SECOND PRELIMINARY AMENDMENT UNDER 37 C.F.R. 1.115

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

INTRODUCTORY COMMENTS

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

Application No.: 14/316,609

Docket No.: AVN-008CN26

AMENDMENTS TO THE CLAIMS

1-7. (Cancelled)

8. (New) An antisense oligonucleotide of 25 bases comprising a base sequence that is 100% complementary to 25 consecutive nucleotides of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the antisense oligonucleotide base sequence comprises at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, wherein the antisense oligonucleotide is chemically linked to a polyethylene glycol chain, and wherein the antisense oligonucleotide specifically hybridizes to the target region to induce exon 53 skipping.

9. (New) A pharmaceutical composition comprising an antisense oligonucleotide of 25 bases comprising a base sequence that is 100% complementary to 25 consecutive nucleotides of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the antisense oligonucleotide base sequence comprises at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, wherein the antisense oligonucleotide is chemically linked to a polyethylene glycol chain, and wherein the antisense oligonucleotide specifically hybridizes to the target region to induce exon 53 skipping, and a pharmaceutically acceptable carrier.

Application No.: 14/316,609

Docket No.: AVN-008CN26

REMARKS

Claim Amendments

Claims 2-7 were pending in the application. Claims 2-7 have been cancelled without disclaimer or prejudice to further prosecution in this or a related application. New claims 8 and 9 have been added. No new matter has been added.

Support for the new claims can be found throughout the claims and specification as originally filed. Specifically, support for the term "morpholino antisense oligonucleotide" can be found at page 17, lines 1-5 (Table 1A) of the specification. Morpholino antisense oligonucleotides have been described in the literature. See, *e.g.*, Summerton, J. and Weller, D. (1997) Morpholino Antisense oligomers: design, preparation, and properties. *Antisense Nucl. Acid Drug Dev.*, 7, 187-195; Heasman, J. (2002) Morpholino Oligos: making sense of antisense? *Dev Biol* 243:209-214; and Gebiski, B. *et al.* (2003) Morpholino antisense oligonucleotide induced dystrophin exon 23 skipping in *mdx* mouse muscle. *Hum. Mol. Gen.* 12(15): 1801-1811. In addition, support for the term "target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69)" can be found throughout the specification as originally filed, for example, at page 21, lines 4-13.

Accordingly, following entry of the foregoing amendment claims 8 and 9 will be pending in the application.

Applicants would like to draw the Examiner's attention to co-pending USSN 14/273,379 (the '379 application), which is directed to substantially similar subject matter as the instant application. To the extent the Examiner is considering issuing an obviousness-type or statutory double patenting rejection of the instant application over the '379 application (or vice-versa), Applicants respectfully request the Examiner to telephonically contact the undersigned prior to issuing such a rejection.

Statement Regarding Interference No. 106,007

Patent Interference No. 106,007 (RES) has been declared between U.S. Patent Nos. 8,455,636 to Wilton *et al.* (the '636 patent) and U.S. Application No. 11/233,495 to van Ommen *et al.* (the '495 application). However, Applicants believe this should not prevent allowance and issuance of the current claims.

Application No.: 14/316,609

Docket No.: AVN-008CN26

The current claims are allowable and patentably distinct over the subject matter of Count 1 of the '007 interference. Count 1 is defined as claim 15 of application '495 application or claim 1 of the '636 patent as shown below:

'495 application

15. An isolated antisense oligonucleotide of 15 to 80 nucleotides comprising at least 15 bases of the sequence cuguugccuccgguucug (SEQ ID NO: 29), wherein said oligonucleotide induces exon 53 skipping in the human dystrophin pre-mRNA, said oligonucleotide comprising a modification selected from the group consisting of: 2'-O-methyl, 2'-O-methyl-phosphorothioate, a morpholine ring, a phosphorodiamidate linkage, a peptide nucleic acid and a locked nucleic acid.

'636 patent

1. An isolated antisense oligonucleotide of 20 to 50 nucleotides in length comprising at least 20 consecutive nucleotides of SEQ ID NO:193, wherein the oligonucleotide specifically hybridizes to an exon 53 target region of the human dystrophin gene inducing exon 53 skipping, and wherein the uracil bases are optionally thymine bases.

Claims 8 and 9 are not anticipated or obvious over the subject matter of Count 1 in view of the state of the prior art. Claim 8 is directed to antisense oligonucleotides having a particular chemical structure resulting from a combination of four features: (i) **25 bases**; (ii) a base sequence that is 100% complementary to a **target region** consisting of 25 consecutive nucleotides **within annealing site H53A(+23+47) and annealing site H53A(+39+69)** and includes at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which **uracil bases are thymine bases**; (iii) a **morpholino** antisense oligonucleotide; and (iv) a **polyethylene glycol chain**.

Claim 9 is directed to a corresponding pharmaceutical composition.

The subject matter of Count 1 fails to describe or otherwise teach or suggest a 25 base morpholino antisense oligonucleotide 100% complementary to a target region consisting of 25 consecutive nucleotides within annealing site H53A(+23+47) and annealing site H53A(+39+69) having at least 20 consecutive bases SEQ ID NO: 193 in which uracil bases are thymine bases, and which is chemically linked to a polyethylene glycol chain.

As such, claims 8 and 9 are in condition for allowance and are patentably distinct over the subject matter of Count 1. The issuance of a Notice of Allowance is thus appropriate.

Application No.: 14/316,609

Docket No.: AVN-008CN26

CONCLUSION

In view of the foregoing, Applicants respectfully submit that the pending claims are in condition for allowance. If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 573-4700. If a fee is due with this response, please charge our Deposit Account No. 12-0080 under Order No. AVN-008CN26, from which the undersigned is authorized to draw.

Dated: August 11, 2014

Respectfully submitted,
Electronic signature: /Amy E. Mandragouras,
Esq./
Amy E. Mandragouras, Esq.
Registration No.: 36,207
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(800) 237-2000 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: January 21, 2015
Electronic Signature for Amy E. Mandragouras, Esq.: /Amy E. Mandragouras, Esq./

Docket No.: AVN-008CN26
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Stephen Donald Wilton *et al.*

Application No.: 14/316,609

Confirmation No.: 4848

Filed: June 26, 2014

Art Unit: 1674

For: ANTISENSE OLIGONUCLEOTIDES FOR
INDUCING EXON SKIPPING
AND METHODS OF USE THEREOF

Examiner: Kimberly Chong

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION
UNDER 37 C.F.R. 1.111

Dear Madam:

In response to the Office Action dated October 21, 2014, please amend the above-identified U.S. patent application as follows:

The Claim Listing is provided on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

Application No.: 14/316,609

Docket No.: AVN-008CN26

CLAIM LISTING

1-7. (Cancelled)

8. **(Previously Presented)** An antisense oligonucleotide of 25 bases comprising a base sequence that is 100% complementary to 25 consecutive nucleotides of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the antisense oligonucleotide base sequence comprises at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, wherein the antisense oligonucleotide is chemically linked to a polyethylene glycol chain, and wherein the antisense oligonucleotide specifically hybridizes to the target region to induce exon 53 skipping.

9. **(Previously Presented)** A pharmaceutical composition comprising an antisense oligonucleotide of 25 bases comprising a base sequence that is 100% complementary to 25 consecutive nucleotides of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the antisense oligonucleotide base sequence comprises at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, wherein the antisense oligonucleotide is chemically linked to a polyethylene glycol chain, and wherein the antisense oligonucleotide specifically hybridizes to the target region to induce exon 53 skipping, and a pharmaceutically acceptable carrier.

Application No.: 14/316,609

Docket No.: AVN-008CN26

REMARKS

Claims 8 and 9 are pending and unamended. The Claim Listing is provided for the Examiner's convenience.

Claims 8 and 9 stand rejected under 35 U.S.C. §103(a) over van Ommen et al. (US Application 20060147952) ("van Ommen et al."), Summerton et al., *Biochemica et Biophysica Acta*, 1489: 141-158 (1999) ("Summerton (1999)"), US 20050048495 ("Baker et al."), US 20030166588 ("Hudziak et al.") and Iversen et al., *Clinical Cancer Research*, 9: 2510-2519 (2003) ("Iversen et al."). Claims 8 and 9 also stand rejected under the doctrine of obviousness-type double patenting over (i) claims 2-5 of co-pending U.S. Application No. 14/273,379; (ii) claims 21-49 of copending Application No. 14/086,859; (iii) claims 1-36 of U.S. Patent No. 8,455,636; and (iv) claims 1-25 of U.S. Patent No. 8,232,384.

Applicants respectfully request reconsideration and withdrawal of the section 103(a) rejection because the Examiner has not established a *prima facie* case of obviousness at least for the reasons established herein. Should the Examiner agree, she is urged to call the undersigned to address any outstanding double patenting rejections prior to mailing a subsequent Office Action to expedite prosecution of this application.

Claim interpretation – meaning of "morpholino antisense oligonucleotide"

Under the subheading "Claim interpretation" the Examiner states, "[w]ith respect to the claimed antisense oligonucleotide comprising thymine bases, Table 1A in the instant specification teach substitution of a U by a T would be using other antisense chemistries such as peptide nucleic acids or morpholinos." (Office Action, at page 4.) The Examiner concludes, "...the limitation wherein the uracil bases are substituted with thymine bases is being interpreted only in the context of the antisense oligonucleotide having chemistries such as morpholinos...The claimed antisense oligonucleotide is a morpholino antisense oligonucleotide wherein the uracil bases are ***thymine bases to represent the morpholino moiety.***" (Office Action, at page 4; emphasis added.)

While Applicants agree that the specification teaches substitution of uracil bases with thymine bases in a morpholino antisense oligonucleotide (e.g., Table 1A), Applicants disagree with the statement that ***thymine bases "represent the morpholino moiety"*** as it is inconsistent with the interpretation that those ordinarily skilled in the art would reach. As evidenced by

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Summerton and Weller (*Antisense and Nucleic Acid Drug Dev*, 7:187-195 (1997) (previously of record)), the term "morpholino antisense oligonucleotide" refers to the chemical structure of the oligonucleotide backbone in which six-membered morpholine rings replace ribose and nucleotides are joined by phosphorodiamidate linkages, and not to the nucleobase sequence itself. The art cited by the Examiner in support of her interpretation is consistent with the established meaning. For example, Hudziak et al. (US 20030166588; cited at page 7 of the Office Action) shows morpholino oligonucleotides as having *either* uracil bases or thymine bases and each sequence description is annotated with a "U" or "T" to reflect the chosen nucleobase. (Hudziak et al. Table 1 at column 10.)

Thus, while the Examiner is correct that the specification describes morpholino antisense oligonucleotides containing thymine nucleobases, Applicants disagree that the term "morpholino antisense oligonucleotide" in and of itself means thymine bases to those of ordinary skill in the art at the time of the invention.

Rejection of Claims 8 and 9 Under 35 U.S.C. §103(a)

Claims 8 and 9 are rejected under 35 U.S.C. §103(a) over van Ommen et al., Summerton (1999), Baker et al., Hudziak et al. and Iversen et al. Applicants respectfully traverse this rejection and request reconsideration and withdrawal based on the following remarks.

The Examiner has failed to establish a prima facie case of obviousness

The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness. (MPEP §2142, 9th Ed.) “The Federal Circuit has stated that ‘rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.’” (*Id.* citing *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006); see also *KSR*, 550 U.S. at 418, 82 USPQ2d at 1396 (quoting Federal Circuit statement with approval).)

“Obviousness is a question of law with underlying factual findings, including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the pertinent art; (3) the differences between the claimed invention and the prior art; and (4) objective evidence such as commercial success, long-felt need, and the failure of others.” (*KSR Int’l Co. V. Teleflex, Inc.*, 550 U.S. 398 (2007) citing *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966).) With respect

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to the third inquiry, to establish a *prima facie* case of obviousness, the Examiner must identify both a reason why a person of ordinary skill in the art would have combined the prior art elements to arrive at the claimed subject matter, and a reason why one of ordinary skill in the art would have considered the outcome predictable. (*KSR Int'l Co. V. Teleflex, Inc.*, 550 U.S. 398 (2007).)

“In cases involving the patentability of a new chemical compound, *prima facie* obviousness under the third *Graham* factor generally turns on the structural similarities and differences between the claimed compound and the prior art compounds.” According to established Federal Circuit precedent, a two-part “lead compound” analysis must be satisfied to establish a *prima facie* case of obviousness. (*Otsuka Pharmaceutical Co. Ltd., v. Sandoz, Inc.*, 678 F.3d 1280 (2012).) To satisfy the lead compound analysis, the Examiner must establish: (1) that one of ordinary skill in the art would have selected the asserted prior art compound as a lead compound for further development, and (2) that the prior art would have motivated one of ordinary skill in the art to modify the lead compound to make the claimed compound with a reasonable expectation of success. (*Id.* at 1291-1292.)

For the reasons below, neither prong of the two part inquiry has been met in the present case. The first prong is not met because one of ordinary skill in the art would have no reason or motivation to select SEQ ID NO: 29 (“h53AON1”) of van Ommen et al. as a lead compound over SEQ ID NO: 4, a 31-mer exon 53 skipping oligonucleotide disclosed in EP 1 160 318 A2 (“Matsuo et al.”; submitted herewith as Exhibit 1). The second prong is not met because, even assuming that one of skill in the art would have selected h53AON1 as a lead compound, there was no reason or motivation to *lengthen* h53AON1 because the experiments by Summerton (1999) relate to either translation suppression or an artificial splicing system, and more relevant human clinical studies at the time of the invention demonstrated efficacy of a **20-mer PMO** at modulating mRNA splicing in humans. Also, there was a significant level of unpredictably associated with selecting a specific antisense oligonucleotide to induce effective exon skipping of human dystrophin pre-mRNA at the time of the invention, and therefore no reasonable expectation of success.

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Docket No.: AVN-008CN26

CONCLUSION

In view of the foregoing, Applicants respectfully submit that the pending claims are in condition for allowance. If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 573-4700. If a fee is due with this response, please charge our Deposit Account No. 12-0080 under Order No. AVN-008CN26, from which the undersigned is authorized to draw.

Dated: January 21, 2015

Respectfully submitted,
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(800) 237-2000 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

Exhibit 11

RESEARCH ARTICLE

Comparative analysis of antisense oligonucleotide analogs for targeted DMD exon 46 skipping in muscle cells

A Aartsma-Rus, WE Kaman, M Bremmer-Bout, AAM Janson, JT den Dunnen, G-JB van Ommen and JCT van Deutekom

Center for Human and Clinical Genetics, Leiden University Medical Center, Wassenaarseweg, AL Leiden, The Netherlands

As small molecule drugs for Duchenne muscular dystrophy (DMD), antisense oligonucleotides (AONs) have been shown to restore the disrupted reading frame of DMD transcripts by inducing specific exon skipping. This allows the synthesis of largely functional Becker muscular dystrophy (BMD)-like dystrophins and potential conversion of severe DMD into milder BMD phenotypes. Thus far we have used 2'-O-methyl phosphorothioate (2OMePS) AONs. Here, we assessed the skipping efficiencies of different AON analogs containing morpholino-phosphorodiamidate, locked nucleic acid (LNA) or peptide nucleic acid (PNA) backbones. In contrast to PNAs and morpholinos, LNAs have not yet been tested as splice modulators. Compared to the most effective 2OMePS AON directed at exon 46, the LNA induced higher skipping levels in

myotubes from a human control (85 versus 20%) and an exon 45 deletion DMD patient (98 versus 75%). The morpholino-induced skipping levels were only 5–6%, whereas the PNA appeared to be ineffective. Further comparative analysis of LNA and 2OMePS AONs containing up to three mismatches revealed that LNAs, while inducing higher skipping efficiencies, show much less sequence specificity. This limitation increases the risk of adverse effects elsewhere in the human genome. Awaiting further improvements in oligochemistry, we thus consider 2OMePS AONs currently the most favorable compounds, at least for targeted DMD exon 46 skipping. Gene Therapy (2004) 11, 1391–1398. doi:10.1038/sj.gt.3302313; Published online 1 July 2004

Keywords: antisense; exon skipping; 2'-O-methyl phosphorothioate; LNA; morpholino; dystrophin

Introduction

Antisense oligonucleotides (AONs) have been reported to modulate pre-mRNA splicing in several studies.¹ For instance, AONs have restored normal splicing by blocking cryptic splice sites,^{2,3} altered the ratio of alternative splicing from malignant to nonmalignant isoforms,⁴ and induced exon inclusion for mutated exons that were otherwise skipped.⁵ In these studies, the AON treatments aimed at the re-establishment of wild-type mRNA. Recently, AONs have alternatively been used to restore the disrupted reading frame of dystrophin mRNAs in Duchenne muscular dystrophy (DMD) gene therapy studies. DMD patients suffer from severe muscle degeneration due to frame-disrupting mutations in the DMD gene that prematurely abort the synthesis of the dystrophin protein.^{6–9} In contrast, mutations in the DMD gene that do not affect the reading frame generate internally deleted but partly functional dystrophins and result in less severe Becker muscular dystrophy (BMD).^{10,11} AON-induced restoration of the DMD reading frame is based on inducing the skipping of specific exons. This was successfully applied in

cultured muscle cells from DMD patients and in the *mdx* mouse model.^{12–19} High exon skipping levels of up to ~90% were achieved, allowing the synthesis of significant levels of BMD-like dystrophins in over 75% of treated cells.¹⁷ These dystrophins located appropriately to the sarcolemma and restored the dystrophin–glycoprotein complex, a strong indication of functional restoration.

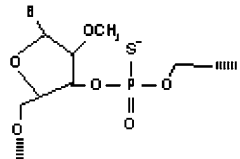
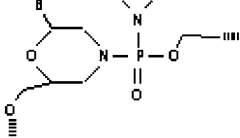
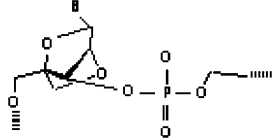
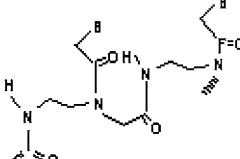
The AONs used in these studies contained 2'-O-methyl modified ribose molecules to render them RNase-H independent, and a full-length 2'-O-methyl phosphorothioate backbone (2OMePS AONs) (Table 1). Although 2OMePS AONs have advantages such as increased resistance to nuclease degradation and increased uptake when compared to phosphodiester AONs, disadvantages are that a phosphorothioate backbone is to some extent cytotoxic, and may elicit an immunogenic response.^{20,21} Recent developments in oligochemistry have provided AONs with different biophysical, biochemical and biological properties based on various modifications to the sugar or the backbone of the nucleotides. Modified AON analogs include morpholino-phosphorodiamidates (morpholinos), locked nucleic acids (LNAs) and peptide nucleic acids (PNAs) (reviewed by Manoharan²¹).

In morpholinos, the sugar phosphate backbone of DNA is replaced by morpholino-phosphorodiamidate oligonucleotides (Table 1).^{22,23} Morpholinos are nontoxic, nuclease resistant,²³ have an increased affinity for RNA and are suggested to disrupt the secondary structure of

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Table 1 Characteristics of the AON analogs used in this study

Backbone	Sequence (5' to 3')	Charge	Length (bp)	Predicted T_m (°C) ^a	Monomer ^b
2'-O-methyl phosphorothioate RNA (2OMePS)	6-FAM-GCUUUUCUUUUAGUUGCUGC	Negative	20	44.9	
Morpholino-phosphorodiamidate DNA (morpholino)	6-FAM-GCTTTTCTTTTAGTTGCTGCTC (linked to sense DNA leash for EPEI transfection)	Uncharged	22	~72–77	
Locked nucleic acid DNA (LNA)	6-FAM-CTTTTAGTTGCTGC	Negative	14	131	
Peptide nucleic acid (PNA)	6-FAM-CTTTTAGTTGCTGC-(Lys) ₄	Positive	14	~61–66	

^a T_m is melting temperature.^bChemical structure of the backbone. B is base (A, C, G, T, U).

RNA.^{1,22} On the other hand, morpholinos are uncharged and therefore difficult to transfect. Nonetheless, reasonable to good efficiencies have been obtained with the scrape loading technique or with ethoxylated poly-ethylenimine (EPEI).^{24,25} Morpholinos have been used, amongst others, to study developmental processes by knocking down genes in zebra fish,²⁶ and to modulate the splicing of the β -globin gene *in vitro* and *in vivo*.^{25,27} Recently, morpholinos have been applied in the *mdx* mouse model to induce the skipping of the mutated exon 23.²⁸ The morpholinos were transfected into the cells in combination with a sense oligo (leash) to allow formation of a cationic lipoplex. This morpholino treatment restored the dystrophin synthesis in *mdx* muscle cells both *in vitro* and *in vivo*.

LNAs are DNA analogs that contain a methylene bridge, which connects the 2' oxygen of ribose with the 4' carbon (Table 1). This bridge results in a locked 3'-endo-conformation, which reduces the conformational flexibility of the ribose.¹ LNAs are nuclease-resistant, nontoxic and have the highest affinity for complementary DNA and RNA yet reported for any DNA analog.^{29,30} This high affinity offers both advantages and disadvantages: LNAs will hybridize very efficiently to their target, but LNAs longer than

15 base pairs (bp) have shown thermally stable self-structuring. Furthermore, full-length LNAs seem to hybridize in a less sequence-specific manner than PNA and 2OMePS AONs.^{29,31} LNAs are negatively charged and cationic lipid polymers are applied for delivery into cells. LNAs have been shown to be potent inhibitors of the expression of a cancer-related gene,³¹ but have not earlier been studied as splicing modulators.

In PNAs, the sugar phosphate backbone of DNA is replaced by an achiral polyamide backbone (Table 1).³² Compared to the 2OMePS backbone, PNAs have a highly increased affinity for DNA and RNA, are suggested to be more sequence-specific, protease- and nuclease-resistant, and nontoxic even at high concentrations.^{1,33} A drawback of the nonionic nature of PNA is its poor water solubility, which makes it complicated to transfect PNAs.^{27,34} Sazani *et al*^{27,35} have recently bypassed this problem by coupling four lysine residues to the C-terminus of PNAs. The cationic nature of the lysine highly improved the water solubility and allowed entrance into the cell and nucleus without transfection reagent. PNAs have successfully been used to modulate the splicing of the murine interleukin-5 receptor- α chain *in vitro* and the β -globin gene *in vitro* and *in vivo*.^{27,35,36}

For future clinical studies, the preferred AON analog induces the highest levels of exon skipping at low levels of cytotoxicity. In this study, we have compared the efficacy, efficiency and applicability of morpholino, LNA and PNA AON analogs to those of a previously described 2OMePS AON^{16,37} specific for DMD exon 46 in both control and patient-derived myotubes.

Results

Comparative analysis of AON analogs in human control myotubes

To determine the binding affinity of the different AON analogs (Table 1) to the target pre-mRNA, a gel mobility shift assay was performed. DNA, 2OMePS, morpholino, LNA and PNA AONs were hybridized to a ³²P-labeled exon 46 RNA fragment (Figure 1a). The DNA, 2OMePS and LNA AONs induced a clear mobility shift, indicating that these analogs are able to bind to the target RNA. No shift was detectable for the morpholino and PNA AONs. This suggested a low affinity of these analogs for the target RNA. However, in previous experiments, we observed that some AONs, while not inducing a notable mobility shift, nevertheless did induce exon skipping (unpublished results). For this reason, we decided to still include the morpholino and PNA AONs in further analyses.

Transfection conditions for the different AON analogs, all carrying a 5' fluorescein group, were optimized in human control myotubes (Figure 1b). Based on the presence of nuclear fluorescent signals, transfection

efficiencies were determined to be typically over 80% for the 2OMePS, morpholino and LNA AONs. The PNA transfection efficiencies were generally lower (~60–70%). Notably, in contrast to the 2OMePS, LNA and PNA AONs, which showed most of the fluorescence within the nucleus, the morpholino was also clearly present in the cytoplasm.

Previous experiments revealed that most efficient skipping levels, as detected by RT-PCR, were obtained at a dose of 500 nM of 2OMePS AONs.¹⁷ Here we show that also LNA and, remarkably, morpholino AONs are effective in inducing exon 46 skipping (Figure 1c). For the morpholino and LNA AONs, concentration series experiments indicated highest skipping efficiencies at doses of 1 μM and 500 nM, respectively. For both analogs, higher concentrations did not result in higher levels of exon 46 skipping (data not shown), but instead induced serious cytotoxic effects for the morpholino AON. Only minimal levels of cytotoxicity were present in myotube cultures transfected with 500 nM of LNA 6 days post-transfection, whereas at that time point severe cell death was observed for 200 nM of the 2OMePS AON (data not shown). Since more units of PEI were applied for LNA transfection, this difference is unlikely due to PEI-induced cytotoxicity. We occasionally observed the skipping of both exons 45 and 46 at low levels in response to high doses of the 2OMePS and LNA AONs (Figure 1c). The PNA did not persistently induce exon 46 skipping, even at a 20 μM dose (data not shown). Exon 46 skipping efficiencies of the different analogs were assessed through quantification of the RT-PCR fragments (Figure 1d). In comparison with the 2OMePS (20%), the

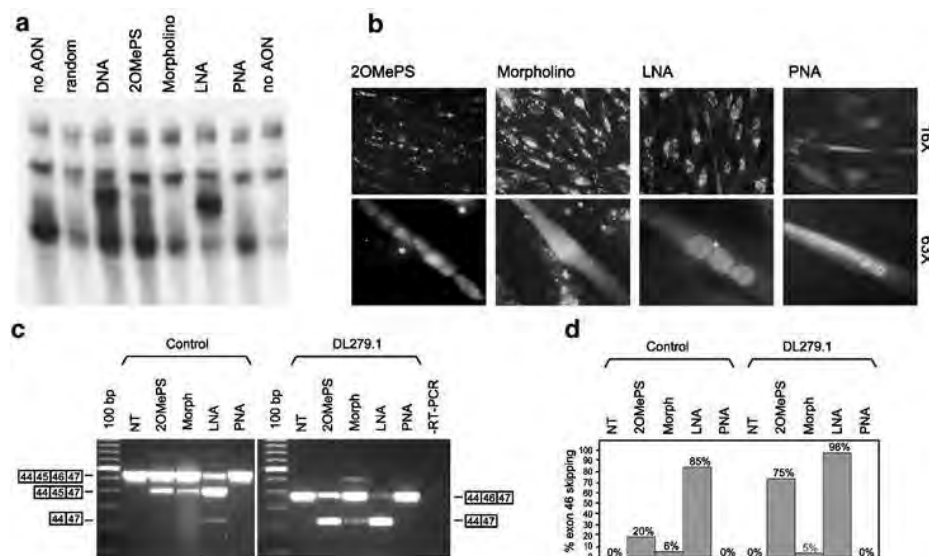


Figure 1 Comparative analysis of DNA, 2OMePS, morpholino, LNA and PNA AONs directed at exon 46. (a) Gel mobility shift assay with a radiolabeled exon 46 RNA fragment hybridized to the different analogs. A clear mobility shift was observed for the AONs with the DNA and LNA backbone, which is less prominent for the 2OMePS AON. No shift was observed for the morpholino, the PNA or a random AON. (b) Transfection of the different AON analogs into control myotubes. Efficiencies of over 80% were obtained for the 2OMePS, the morpholino and the LNA AONs, whereas for the PNAs efficiencies were 60–70%. In contrast to the other AON analogs, the morpholino was also abundant in the cytoplasm. (c) RT-PCR analysis of dystrophin mRNA fragments from unaffected control and DMD patient-derived (DL279.1) myotubes following transfection of the different AON analogs. With the exception of the PNA, exon 46 skipping was detected for each analog, both in patient and control myotubes. The precise skipping of exon 46 was confirmed by sequence analysis (data not shown). The DNA size marker is 100 bp. (d) Quantification of RT-PCR products. The percentage of exon 46 skipping was determined by the ratio of the shorter fragment to the total of transcript fragments, and is shown above each column. The LNA induced highest levels of exon skipping in both unaffected control (85%) and patient-derived (98%) myotubes. The 2OMePS AON was remarkably more efficient in the patient-derived myotubes when compared to the control (75 versus 20%, respectively). The morpholino was only moderately effective (5–6%) both in control and patient-derived myotubes.

LNA AON was much more efficient (85%), whereas the skipping levels for the morpholino were not higher than 6%.

Comparative analysis of AON analogs in patient-derived myotubes

We have previously observed that 2OMePS AONs induced higher levels of exon skipping in patient-derived myotubes when compared to control samples.^{16,17} This effect was confirmed here in myotubes derived from a DMD patient (DL279.1) affected by an exon 45 deletion. For this patient, exon 46 skipping generates in-frame transcripts. We observed 75% exon 46 skipping in DL279.1 *versus* 20% in control myotubes for the 2OMePS AON (Figure 1c and d). The LNA and morpholino AONs were also able to induce exon skipping (98 and 5%, respectively), whereas the PNA was not (Figure 1c and d). Similar to the 2OMePS AON, the LNA showed higher skipping levels in DL279.1 when compared to those in control myotubes (98 *versus* 85%). This effect however was not significant for the morpholino or PNA AONs.

Dose effects and sequence specificity of LNA versus 2OMePS AONs

Since the LNAs induced highest skipping levels, we performed a concentration series to determine the minimally effective dose. RT-PCR (Figure 2a) and quantitative analysis (Figure 2b) showed that in human control myotubes the efficiency dropped markedly from 97 to 30% at doses lower than 500 nM, and that very low

levels of exon 46 skipping (<1%) were detectable at 100 nM. In patient-derived myotubes, however, the levels of exon skipping decreased more gradually at lower doses, with exon 46 skipping levels of 86% detectable at a dose of 300 nM, and still a significant level (10%) at 100 nM (Figure 2a and b).

We then analyzed the sequence specificity of LNA and 2OMePS AONs in patient myotubes (since they showed higher skipping levels than control myotubes). We studied five different LNAs, which contain one or two mismatches at the 5', 3' or center position (LNAm1–5; Figure 3a), and one LNA that was shifted in the 3' direction of exon 46 (LNA9; Figure 3a). A gel mobility shift assay revealed that all LNAs were able to bind to the target pre-mRNA (data not shown). The LNAs were then transfected into patient myotubes at a dose of 500 nM. RT-PCR analysis (Figure 3b) and quantification (Figure 3c) showed that the LNAs containing one or two mismatches at the 3' end (LNAm1 and LNAm4) were able to induce exon 46 skipping at high levels, comparable to the original 100% complementary LNA8 (71–94 *versus* 100%), suggesting that LNAs have a poor sequence specificity. The LNA containing a single mismatch in the center (LNAm2) induced only low levels (8%) of exon skipping, whereas the LNA containing two mismatches in the center (LNAm5) and the 5' mismatched LNA (LNAm3) did not induce any skipping. LNA9 did not induce exon 46 skipping, even though it is completely homologous to exon 46. We also assessed the effects of lower concentrations of the 3' mismatch-containing LNAs, and observed exon skipping at comparably lower levels as with LNA8 (data not shown).

We similarly tested five different 2OMePS oligos containing up to three mismatches when compared to the original 2OMePS oligo in patient myotubes (2OMePSmm1–5; Figure 3d). In contrast to the LNAs, the 2OMePS AONs containing one or two mismatches at the 3' end (2OMePSmm1–2) induced exon 46 skipping at significantly reduced levels when compared to the original oligo (7–17 *versus* 48%) (Figure 3e and f). The oligos containing three mismatches at the 3' end (2OMePSmm3) or one at the 5' end (2OMePSmm4) induced only barely detectable levels (1–2%) of exon skipping, whereas the 2OMePS containing three mismatches dispersed throughout the oligo (2OMePSmm5) was completely unable to induce exon skipping. Transfection experiments and RT-PCR analyses of the mismatched 2OMePS AONs and LNAs were repeated several times and showed reproducible efficiencies. Our results indicate that the 2OMePS AONs have a higher sequence specificity than the LNAs.

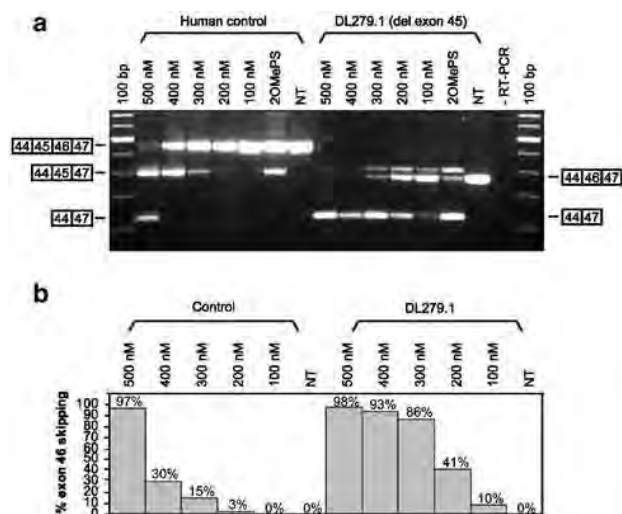


Figure 2 Concentration series of LNA8 in control and patient-derived (DL279.1) myotube cultures. (a) RT-PCR analysis of dystrophin mRNA fragments. Significant levels of exon 46 skipping were observed at each dose tested for the patient (DL279.1), whereas only low levels were detected for a dose of 200 and 100 nM in the human control. In control myotubes, skipping of both exons 45 and 46 was sometimes observed at the highest doses (400 and 500 nM). For some DL279.1 fragments, products slightly larger than the wild-type fragments can be observed. This is due to heteroduplex formation of first and second PCR products. The DNA size marker is 100 bp. (b) Quantification of the RT-PCR fragments showed that the levels of exon skipping decrease considerably at doses below 500 nM for the human control (from 97% at 500 nM to 30% at 400 nM and below 1% at 100 nM), but remain high (86%) at a dose of 300 nM for the patient and still significant (10%) at a 100 nM concentration.

Discussion

In our previous studies on antisense-induced exon skipping, 2OMePS AONs were applied.^{16,17,37} In this study, additional AON analogs were tested for their efficacy, efficiency and applicability in inducing DMD exon 46 skipping in control and DMD patient-derived myotube cultures. Towards future clinical trials, the most optimal AON analog should induce high levels of exon skipping, but also be nontoxic, easy to deliver and, preferably, relatively inexpensive. Out of the four

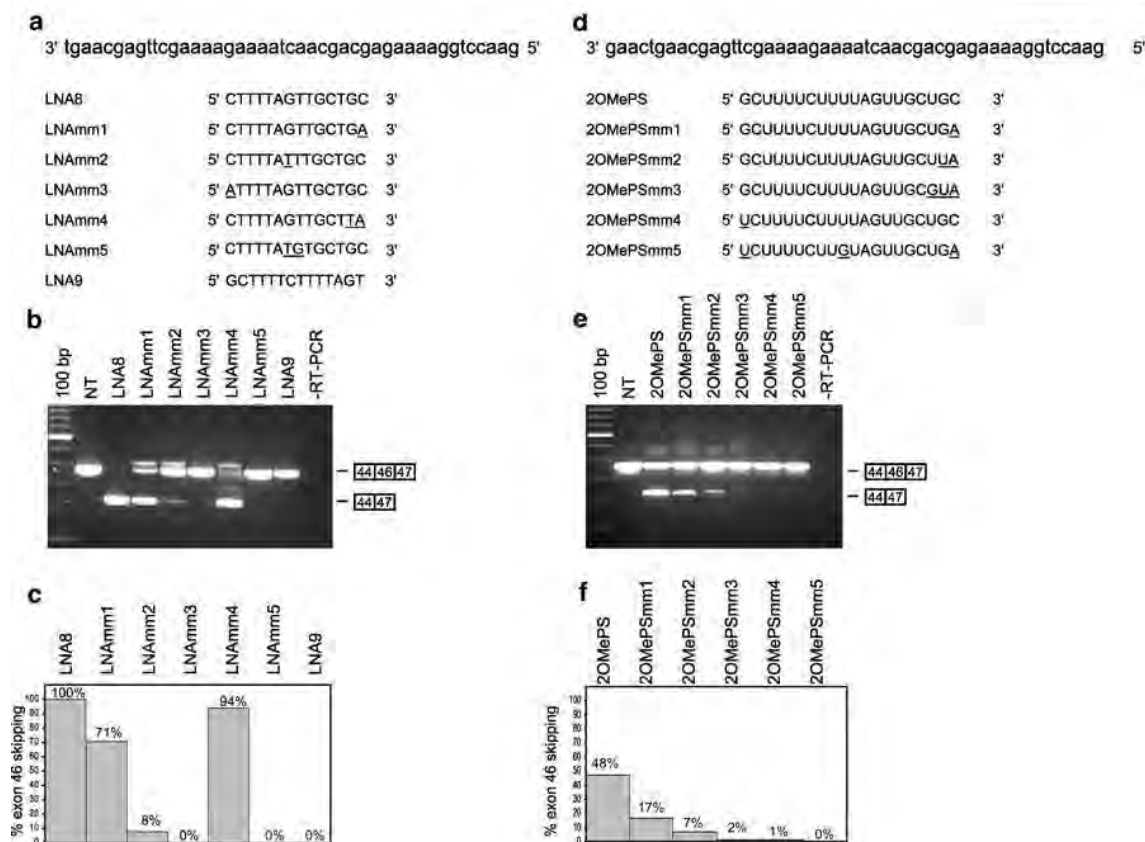


Figure 3 Analysis of LNA and 2OMePS AONs containing mismatches in patient-derived myotubes. (a) Sequences of the different LNAs aligned to their target sequence in exon 46 (depicted at the top from 3' to 5'). Mismatches to the target sequence are underlined. LNA9 is shifted to the 3' side of the target sequence when compared with the original LNA (LNA8) and is completely homologous to the target sequence. (b) RT-PCR analysis and (c) quantification of dystrophin mRNA fragments from LNA-treated patient (DL279.1) myotubes. The LNAs containing mismatches in the 3' end (LNAmm1 and 4) induced exon 46 skipping to levels that are comparable to those induced by the original LNA (71–94 versus 100%). The mismatches in the 5' or central part (LNAmm2, 3 and 5) seriously reduced the skipping capacity (<8%). Fragments slightly larger than the wild-type band were observed. This is due to heteroduplex formation between first and second PCR products. The DNA size marker is 100 bp. (d) Sequences of the different 2OMePS AONs aligned to the target sequence in exon 46 (depicted at the top from 3' to 5'). Mismatches to the target sequence are underlined. (e) RT-PCR analysis and (f) quantification of dystrophin mRNA fragments from 2OMePS-treated patient (DL279.1) myotubes. The presence of one mismatch at the 3' side (2OMePSmm1) already results in an over 2.5-fold reduction in exon skipping levels, while even more reduced levels are found for the AONs containing two and three mismatches (2OMePSmm2 and 3). Virtually no skipping was induced by the AONs that contain a single mismatch at the 5' part (2OMePSmm4) or a single mismatch in the 3', central as well as the 5' part (2OMePSmm5) of the oligo. The DNA size marker is 100 bp.

alternative analogs tested here, only LNAs induced higher levels of exon skipping when compared to 2OMePS AONs. The morpholino was less efficient in both patient and control myotubes, whereas the PNA was completely ineffective. For both the LNA and 2OMePS AONs, the levels of exon skipping were higher in patient-derived cells than in control cells. We have observed this effect previously in cells from other DMD patients¹⁷ and hypothesize that it is due to nonsense-mediated RNA decay (NMD), which will selectively target the out-of-frame skip-product in control myotubes and thereby negatively influence the relative amount of skip-product. In patient-derived cells, the original out-of-frame mRNA is subject to NMD, whereas the in-frame skip-product is not. Another explanation may be that AON-mediated exon skipping is actually enhanced in the patient due to the presence of the deletion that already perturbed local splicing. The levels of morpholino-induced exon skipping were comparable in the patient and control samples (6 versus 5%). Although we

expected higher levels for the patient, the morpholino-induced skipping levels are so low that it may be difficult to detect significant differences. It is also possible that the morpholino has a lower affinity for pre-mRNA of the patient than for wild-type dystrophin pre-mRNA, as a consequence of the exon 45 deletion (eg due to an altered secondary structure).

The gel mobility assay showed no shift for the morpholino and PNA AONs, but despite its apparent low affinity for the target pre-mRNA, the morpholino was able to induce low levels of exon 46 skipping. This may be explained by the fact that the morpholino was hybridized to a sense DNA oligo (a 'leash' allowing EPEI-coupled transfection), which may have interfered with the proper hybridization to the target RNA fragment. After transfection into cells, the leash detaches from the morpholino, which is then free to hybridize to the target RNA. Schmajuk *et al*²⁵ found that morpholinos were more effective in restoring the wild-type splicing of the β -globin gene when compared to 2OMePS AONs.

However, since we observed fluorescence in both the cytoplasm and the nucleus after morpholino transfections, the low levels of exon skipping may be the result of poor nuclear uptake rather than the low efficiency of the morpholino. Further optimization of the morpholino itself and the leash required for EPEI transfection may thus increase the levels of exon 46 skipping. Indeed, Gebiski *et al*²⁸ have recently shown that levels of exon 23 skipping in the *mdx* mouse varied when different leashes were used.

Even though the sequences of the PNA and the LNA analogs are completely identical, the LNA induces high levels of exon 46 skipping, whereas the PNA induces no skipping at all. PNA oligos have been reported to induce higher levels of correctly spliced β -globin mRNA than morpholino and 2OMePS analogs.^{27,35} This indicates that PNA is in fact able to modulate splicing and suggests that the lack of exon 46 skipping in our experiments may result from the inability of our PNA to bind to the specific target RNA sequence, or a poor stability of the PNA–RNA complex. Further experiments may identify PNAs with higher binding affinities to exon 46 target sequences.

LNAs are relatively new AON analogs that have thus far only been applied to inhibit the expression of target genes.^{29,31} We show here that LNAs are also very potent modulators of pre-mRNA splicing. In a series of experiments, the LNA induced exon 46 skipping in 85% of control transcripts and in 98% of transcripts from a DMD patient carrying an exon 45 deletion. In comparison, the 2OMePS AONs previously used induced 20% exon 46 skipping in control cells and 75% skipping in exon 45-deleted transcripts. Notably, the LNA also seemed to be less toxic than 2OMePS AONs.

Based on these results, LNA in principle may be a promising alternative for antisense-induced exon skipping studies. Unfortunately however, due to the shorter sequence (14-mer), it shows complete homology to several other sequences in the human genome. Most of these were located within noncoding regions, either in or near genes. Increasing the length of the LNA to enhance specificity is problematic, since 15-mer LNAs have been reported to show thermally stable self-structuring.²⁹ In addition, our results with LNAs containing mismatches show that LNAs with one or two mismatches in the 3' part of the AON were almost as potent as the specific LNA. This suggests that the binding of only 12 bp is enough to induce the skipping of exon 46, which is not surprising, given the extremely high melting temperatures of LNA (predicted to be 131°C for our 14-mer LNA; Table 1). It does, however, imply that our exon 46-specific LNA may also adversely bind to other sequences in the human genome that contain these 12 bp. The LNA may bind to even shorter sequences, since, for instance, a 7-mer still has a predicted melting temperature of over 60°C. Furthermore, we have recently injected the human LNA into mouse muscle. With one mismatch in the center of the AON when compared to the mouse sequence, the human LNA yet induced skipping of the murine exon 46, even at low concentrations (data not shown). This again underlined the potential risk for nonsequence-specific effects associated with LNAs. To decrease the affinity for RNA, chimeric LNA/2'-O-methyl RNA oligos have been generated.³⁸ These chimeras have lower melting temperatures than full-

length LNA, but still have higher affinities for RNA than 2OMePS AONs. In fact, chimeric LNAs have been shown to block transcription of the HIV-1 transactivating responsive element *in vitro*.³⁸

Our results with mismatched 2OMePS AONs show that these analogs are more sequence specific than the LNAs. The presence of one mismatch at the 3' end results in an almost three-fold decrease of exon 46 skipping levels, whereas no exon skipping was left with 2OMePS AONs containing three 3' mismatches. One might have anticipated a greater effect of a single mismatch in the shorter 14-mer LNA than in the longer 20-mer 2OMePS AON. However, the opposite was found true for the 3' end. Furthermore, for both the LNA and the 2OMePS analogs, mismatches at the 3' end induced higher levels of exon 46 skipping than those at the 5' end or in the center of the AONs. This suggests that, whether or not due to the presence of the fluorescent label, mismatches at the 5' end reduced the affinity for the target RNA to a larger extent.

By choosing other target sequences within the exon, the levels of exon 46 skipping may be optimized for the PNA and morpholino AONs. Similarly, these analogs may be more effective in inducing the skipping of other DMD exons. This would agree with reports showing that morpholinos and PNAs are more efficient than 2OMePS analogs in inducing the skipping of murine exon 23 or correcting aberrant β -globin splicing, respectively.^{27,28,35} In other words, for each different target sequence, the best choice of oligochemistry may vary. The relatively wide mutation spectrum in DMD requires the design of a series of efficient AONs targeting different exons. In that respect, we have obtained good results with 2OMePS AONs. By designing only two AONs per exon, we identified at least one efficient AON for 14 out of the 17 exons targeted³⁷ (unpublished results). Whether or not AON design is as simple using other analogs remains to be established.

Materials and methods

AONs and primers

The characteristics of the AON analogs in this study are reviewed in Table 1. The 2OMePS AONs (sequences shown in Figure 3d; Eurogentec, Belgium) have a full-length phosphorothioate backbone and 2'-O-methyl modified ribose molecules, as previously described.^{16,37} The morpholino (Gene-Tools, USA) has a morpholino-phosphoroamidate backbone and is linked to a sense DNA oligo for EPEI transfection. The LNAs (sequences shown in Figure 3a; Prologo, France) have full-length LNA backbones. The PNA (Eurogentec, Belgium) has a PNA backbone and four lysine residues at the C-terminal end to improve water solubility and facilitate transfection into cells. All AONs contain a 5' fluorescein group (6-FAM) to confirm nuclear localization of the AONs following transfection. Note that we have previously shown that labeled and unlabeled 2OMePS AONs were equally efficient in restoring dystrophin synthesis in DMD patient-derived myotube cultures.^{17,18}

Gel mobility shift assay

Human dystrophin exon 46 RNA was *in vitro* transcribed as described previously.¹⁶ The binding affinity of the

individual AONs (at a dose of 0.5 pmol) was determined by overnight incubation in a hybridization buffer (1 mM Tris-HCl pH 7.4, 50 mM NaCl, 5 mM MgCl₂) at 37°C, followed by 8% PAGE and PhosphorImager analysis (Molecular Dynamics).

Myogenic cell cultures and transfection

Primary human myoblasts from an unaffected control and a DMD patient (DL279.1; carrying a deletion of exon 45) were isolated from muscle biopsies and cultured as described previously.³⁷ Myotubes were obtained from confluent myoblast cultures, following 7–14 days of serum deprivation. For the 2OMePS and LNA AONs, PEI was used for transfection, according to the manufacturer's instructions (ExGen500; MBI Fermentas), with 3.5 µl PEI per µg of transfected AON (ie 12.9 µl (70 µM) for the 2OMePS and 9.2 µl (50 µM) for the LNA oligo). EPEI was used to transfect the morpholino AON according to the manufacturer's instructions, with 1 µl of 200 µM EPEI (Gene-Tools, USA) for each 200 nmol of morpholino. PNAs were applied to 1 ml culture medium, without any transfection reagent. At 3 h post-transfection, 2 ml medium was added.

RNA isolation, RT-PCR and sequence analysis

RNA isolation, RT-PCR analysis and direct sequencing were performed as described.¹⁷ Primary PCRs included primers in exon 43 (CCTGTGGAAAGGGTGAAGC) and 48 (CTGAACGTCAAATGGTCTTC); nested PCRs included primers in exon 44 (CGATTTGACAGATCTGTT GAG) and 47 (GAGCACTTACAAGCACGGG). All primers were synthesized by Eurogentec (Belgium). For quantification, the skip-products were analyzed using the DNA 1000 LabChip[®] Kit on the Agilent 2100 bioanalyzer (Agilent Technologies, USA).

Acknowledgements

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Exhibit 12

**THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

NIPPON SHINYAKU CO., LTD.,

Plaintiff,

v.

SAREPTA THERAPEUTICS, INC.,

Defendant.

C.A. No. 21-1015 (JLH)

SAREPTA THERAPEUTICS, INC. and
THE UNIVERSITY OF WESTERN
AUSTRALIA,

Defendant/Counter-Plaintiffs,

v.

NIPPON SHINYAKU CO., LTD.
and NS PHARMA, INC.

Plaintiff/Counter-Defendants.

SUPPLEMENTAL REBUTTAL EXPERT REPORT OF STEVEN F. DOWDY, Ph.D.

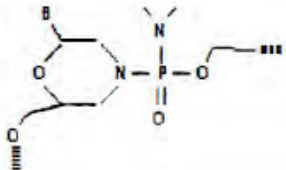
21. Moreover, Dr. Hastings' contention that the Wilton Patents may encompass "billions" of ASOs is both flawed and unsupported. Dr. Hastings' claim scope calculation is premised on three types of possible chemical variations: intersubunit linkages (Hastings Supp. Rep. ¶¶61-65, 80), nucleobase modifications (Hastings Supp. Rep. ¶¶51-54, 76-78), and 5' - or 3' - end modifications (Hastings Supp. Rep. ¶¶55-60, 79). Each is addressed below.

i. Intersubunit Linkages

22. Relying on Dr. Wood, Dr. Hastings argues that "the term 'morpholino' is broader than just a *phosphorodiamidate* morpholino oligomer ('PMO') and encompasses ASOs with different types of morpholino intersubunit linkages." Hastings Supp. Rep. ¶61 (emphasis in original). Dr. Hastings estimates that "at least fifty-six (56) chemically distinct types of morpholino inter-nucleotide linkages" are allowed by the claims of the Wilton Patents, and states that different intersubunit linkages can be used within a single ASO. Hastings Supp. Rep. ¶80. But Dr. Hastings ignores the state of the art and the prosecution history of the Wilton Patents. In view of this evidence, a POSA would have understood that, consistent with the plain and ordinary meaning of the term, the claimed "morpholino antisense oligonucleotide" refers to a *phosphorodiamidate morpholino oligomer*, i.e., an ASO wherein each ribose moiety is replaced with a morpholine moiety, and each phosphodiester linkage is replaced with a phosphorodiamidate linkage. *See* Dowdy Op. Rep. ¶83.

23. Numerous references published before June 2005, including those cited by Dr. Hastings and submitted to the Patent Office, show that the term "morpholino" refers to a phosphorodiamidate morpholino oligomer. As summarized below, each of these prior art references uses the term "morpholino" antisense oligonucleotide to refer to a PMO (with exclusively phosphorodiamidate linkages), as indicated below.

<p>Summerton 1997</p>	<div data-bbox="672 201 1221 835" data-label="Chemical-Block"> <p>B = adenine, cytosine, guanine, uracil</p> </div> <p>FIG. 2. Morpholino oligo structure. (Summerton 1997, Fig. 2)</p>
<p>Heasman 2002</p>	<p>“Morpholino oligos were designed specifically to overcome many of the limitations of regular DNA oligos (Gene Tools, LLC). Recognizing that RNase H-mediated degradation was not the only efficient way to prevent translation, Summerton and Weller (1997) developed a DNA analog that acts by blocking translation. This analog has the riboside moiety of each subunit converted to a morpholine moiety (morpholine = C₄H₉NO), and uses a phosphorodiamidate intersubunit linkage instead of phosphodiester linkages (see Summerton and Weller, 1997 for a detailed description).” (Heasman 2002, 210) (emphasis added).</p>
<p>Gebski 2003</p>	<p>“One chemistry that is gaining wide recognition for use in antisense applications is the morpholino oligonucleotide developed by Summerton and Weller (12). These authors developed the morpholino structural type with the intention that this chemistry could provide several advantages in the clinical application(s) of antisense therapeutics, such as strong nucleic acid binding, resistance to nucleases, minimal nonantisense effects, high aqueous solubility and relatively low synthesis costs (12).” (Gebski 2003, 1802) (emphasis added).</p> <p>“12. Summerton, J. and Weller, D. (1997) Morpholino antisense oligomers: design, preparation, and properties. <i>Antisense Nucl. Acid Drug Dev.</i>, 7, 187-195.” (<i>Id.</i>, 1810).</p>

Aartsma-Rus 2004	Table 1 Characteristics of the AON analogs used in this study
	BackboneMonomer ^b
	Morpholino-phosphorodiamidate DNA (morpholino)
	<div>***</div> <div></div>

24. These references, all of which published before June 28, 2005 and three of which were cited specifically to the Patent Office as support for the claim term, demonstrate that a POSA reading the Wilton Patents would have understood the term “morpholino antisense oligonucleotide” to refer to a PMO, not an oligomer with different intersubunit linkages.

25. This was explained to the United States Patent Office during prosecution of the '851 Patent. In the Preliminary Amendment presenting claims that ultimately issued as the claims of the '851 Patent, the Applicant stated that “support for the term ‘morpholino antisense oligonucleotide’ can be found at page 17, lines 1-5 (Table 1A) of the specification.” '851 Prosecution History, SRPT-VYDS-0003101. The Applicant further stated that “[m]orpholino antisense oligonucleotides have been described in the literature” and cited three exemplary prior art references as support, each of which are discussed in the table above. *Id.* (citing Summerton 1997, Heasman 2002, and Gebiski 2003).

26. The prosecution history of U.S. Patent No. 9,024,007, which claims priority to the same patent application as the '851 Patent and other asserted Wilton Patents (International Patent Application No. PCT/AU2005/000943), further reinforces this. On August 11, 2014, the Applicant submitted a set of claims reciting the term “morpholino antisense oligonucleotide.” The Applicant explained that the term was described in prior art including Summerton 1997, Heasman



DATE: August 14, 2024



By: _____
Steven F. Dowdy, Ph.D.

Exhibit 13



REVIEW

Morpholino Oligos: Making Sense of Antisense?

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Since morpholino oligos were first introduced as a means to inhibit gene function in embryos, in the Spring of 2000, they have been tested in a range of model organisms, including sea urchin, ascidian, zebrafish, frog, chick, and mouse. This review surveys the results of these studies and examines the successes and limitations of the approach for targeting maternal and zygotic gene function. The evidence so far suggests that, with careful controls, morpholinos provide a relatively simple and rapid method to study gene function. © 2002 Elsevier Science (USA)

Key Words: antisense; morpholino; embryo.

INTRODUCTION

The demand for knockout technology in developmental biology is rising exponentially as genes of interest emerge at an ever-increasing rate from genomic studies, and therefore the central task becomes to relate genes to functions. Historically, lengthy mutant screens in *Drosophila*, and *Caenorhabditis elegans* have provided us with most of our knowledge of gene function in development, but reverse genetic approaches have become increasingly attractive, because they offer the possibility of taking interesting candidate genes from genomic databases and speedily identifying their roles.

Against this background, it is not surprising that the introduction of a new loss-of-function technology has been met with enthusiasm by developmental biologists. Morpholino antisense oligos were first developed for clinical therapeutic applications, where previous antisense approaches had proven seriously flawed (Summerton and Weller, 1997). They were first introduced into developmental biology early in 2000 (Heasman *et al.*, 2000) and have since been used by researchers in a range of model organisms, including sea urchin (Howard *et al.*, 2001), *Ciona savignyi* (Satou *et al.*, 2001), *Xenopus laevis* (Audic *et al.*, 2001; Schweickert *et al.*, 2001; Sumanas *et al.*, 2001), *Xenopus tropicalis* (Nutt *et al.*, 2001), zebrafish (Nasevicius and Ekker, 2000; Bauer *et al.*, 2001; Ross *et al.*, 2001;

Segawa *et al.*, 2001; Shepherd *et al.*, 2001; Yang *et al.*, 2001, and 19 papers in *Genesis* 30, July 30, 2001), chick (Kos *et al.*, 2001), and mouse (Coonrod *et al.*, 2001). In the summer of 2001, an entire issue of the journal "Genesis" was dedicated to articles studying gene function in development using this technique. As the first wave of usage is well underway, the aim of this review is to survey the successes and limitations of this latest loss-of-function tool.

Morpholino Oligos Block Messenger mRNA Translation

First generation antisense oligos were designed as short stretches of DNA (18–22 mers) that form RNA–DNA hybrids with the target mRNA and act as substrates for RNase H to degrade the mRNA (Cazenave *et al.*, 1989). The target mRNA is cleaved by RNase H, and the fragments are subsequently broken down by nuclease activity. DNA oligos have had very limited applicability in developmental studies, both because they have nonspecific toxic side effects, (Heasman *et al.*, 1991) and because degraded mRNAs are continually replaced by new transcription, making continued treatment with oligo a necessity. The one exception to this general case is that of loss-of-function studies of maternal genes in *Xenopus* embryos (Zuck *et al.*, 1998). Here, antisense oligos are injected into the oocyte, which is then cultured for several days before fertilization. The oligos degrade the target mRNA, and are themselves broken down, so that they have no toxic effect on the

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embryos after fertilization. This, together with the fact that there is no transcription in the developing *Xenopus* embryo until the 4000-cell stage, makes DNA oligos a feasible approach for functional analysis in this restricted area (Heasman *et al.*, 1994; Zhang *et al.*, 1998).

Morpholino oligos were designed specifically to overcome many of the limitations of regular DNA oligos (Gene Tools, LLC). Recognizing that RNase H-mediated degradation was not the only efficient way to prevent translation, Summerton and Weller (1997) developed a DNA analog that acts by blocking translation. This analog has the riboside moiety of each subunit converted to a morpholine moiety (morpholine = C₄H₉NO), and uses a phosphorodiamidate intersubunit linkage instead of phosphodiester linkages (see Summerton and Weller, 1997 for a detailed description). Morpholino oligos only block translation when they are designed to be complementary to the 5' leader sequences, or to the first 25 bases 3' to the AUG translational start site, and it is presumed that they act by preventing ribosomes from binding. In cell-free translation experiments, they were shown to have greater efficiency and specificity than other antisense oligos, and tests showed excellent solubility and stability characteristics (Summerton, 1999). Because the 5'UTR is less conserved than coding regions, the chance of the morpholino oligo blocking incorrect mRNAs nonspecifically is less than for traditional oligos. Their increased specificity also derives from the fact that translation is not blocked when there are several mismatched nucleotides. For most effective RNA targeting, oligos are recommended to be 25 mers in length, and have little self-complementarity (four or fewer contiguous intrastand base pairs). Water solubility is maintained by using less than 36% guanine content and no runs of more than triplets of GGG (Gene Tools, LLC).

The next critical phase for the development of morpholino oligos as loss-of-function tools in developmental studies has been to test them in embryos.

Morpholino Injections Phenocopy Known Mutants

The critical questions to answer for testing morpholino oligos' reliability as loss-of-function tools are, first, whether they mimic the phenotypes of known mutants, and if so, how reproducible or penetrant the effects are. Of equal importance in the development of morpholinos as tools to study novel gene function is to recognize toxic side effects of oligo injection, to avoid the trap of confusing those effects with those caused by true loss-of-function. A third important consideration in deciding whether morpholinos could be useful in studying genes acting during organogenesis is how long after injection they actively block translation.

Most published work to date testing morpholinos has been carried out on zebrafish embryos, where many mutants are available that have defined the roles of genes important for development. In fish embryos, oligos are

TABLE 1

Genetic mutant	Reference
<i>swirl, snailhouse, somitabun, minifin, silberblick, pipetail</i>	Lele <i>et al.</i> , 2001
<i>no tail, chordin, one-eyed-pinhead, nacre, sparse</i>	Nasevicius and Ekker, 2000
<i>cyclops</i>	Karlen and Rebagliati, 2001
<i>snailhouse, swirl</i>	Imai and Talbot, 2001
<i>squint, one-eyed pinhead, notail</i>	Feldman and Stemple, 2001
<i>sucker/edn1</i>	Miller and Kimmel, 2001
<i>colourless</i>	Dutton <i>et al.</i> , 2001
<i>lost-a-fin</i>	Bauer <i>et al.</i> , 2001

typically injected at the one- to four-cell stage into the cytoplasm or yolk. Table 1 is a partial list of the genes that have been studied by their loss-of-function both in mutants and in morpholino-injected embryos. Clearly, this represents the most positive view of the success of the morpholino approach since injections which do not phenocopy mutants are much less likely to be published. However, in two papers, the success rate of tested genes was reported. Lele *et al.* (2000) report a 100% success rate for 7/7 genes (*swirl, snailhouse, somitabun, minifin, silberblick, pipetail*, and *lost-a-fin*), while Nasevicius and Ekker (2000) report a 100% success rate for all nine genes studied (including *no tail, chordin, one-eyed-pinhead, nacre*, and *sparse*). This provides very substantial encouragement for pursuing this antisense approach.

However, these data also highlight several characteristics of morpholino-induced phenotypes, most importantly, the variability of the severity of the phenotypes and the non-specific side effects caused by oligo injection.

Variability in the Severity of Phenotypes

An important consideration in the use of morpholinos is the extent to which they give consistent results when one dose is injected into a group of embryos. Importantly, injections of a morpholino complementary to *GFP* mRNA reduced GFP protein expression in all cells of fish expressing a *GFP* transgene, indicating that, at least in this case, the morpholino spread evenly throughout the zebrafish embryo after injection (Nasevicius and Ekker, 2000). In some cases, extremely consistent results have been reported for oligo effectiveness. For example, a *bmp7* oligo causes extreme dorsalization at final concentrations of 1 and 4 μ M in 81 and 93% of cases, respectively (Imai and Talbot, 2001). A 5.4 μ M final concentration of *chordin* oligo phenocopies the *chordin* null mutant embryo in more than 75% of cases (Nasevicius and Ekker, 2000), and *no tail* is phenocopied in 98% (Nasevicius and Ekker, 2000; using 10.8 μ M) and 100% of cases (Feldman and Stemple, 2001; using 2.1–8.6 μ M final concentration). For *no tail*, this correlates with a complete lack of protein on a Western blot (Nasevicius and Ekker, 2000).

For other targets, the results are much more variable. Smad 5 depletions, caused by a 6.5-ng dose, giving a final concentration of 7.8 μ M, resulted in 24% of embryos with a weak phenotype compared with the genetic mutant, *somitabun*, while 49% resembled the mutant and 27% had stronger effects (Lele *et al.*, 2001). Higher doses than 6.5 ng caused nonspecific effects. *One-eyed-pinhead* morpholino-injected embryos showed the expected phenotype in only 43% of cases (10.8 μ M), and the rest of the embryos were normal (Nasevicius and Ekker, 2000). Thus, the potential variability in penetrance of the morpholino is an important consideration for interpreting data. While, in some instances, a graded series of phenotypes equivalent to an allelic series may be very beneficial for functional analyses, when novel genes are being studied, it may add to the uncertainty of what is real and what is a nonspecific side effect. This will be particularly true if the toxic dose is close to the effective dose.

A second variability to keep in mind when comparing morpholino-induced mutant phenotypes and genetic mutations, is that the morpholino derived mutant might lie anywhere on the entire spectrum of phenotypes caused by complete depletion of protein to only a slight reduction in protein levels. The degree of depletion will depend on many parameters, as well as oligo concentration, including the amount and stability of the protein that is present at the time of oligo introduction, the amount of diffusion, the localization of the targeted mRNA, and the amount of new transcription. In making meaningful comparisons with genetic phenotypes, it is also helpful to compare the morpholino phenotype with both null mutations and hypomorphic mutations. It is clearly possible for morpholinos to cause more severe phenotypes than hypomorphs, or null alleles that only affect zygotic gene products (e.g., Bauer *et al.*, 2001).

Side Effects of Morpholino Oligos

Since morpholino oligos were developed for delivery by scrape-loading into cells in tissue culture, no guidelines suitable for embryo injection were commercially available. Thus, researchers routinely injected a series of increasing doses of oligo into zebrafish embryos and examined them for both expected and unexpected phenotypes. In typical experiments, 1 nl of a range of concentration of oligo (0.1–0.8 mM; with a final concentration of 1–8 μ M) was injected into the yolk of genetically wild-type embryos at the one-cell stage. Several authors report that, at the high end of this range, nonspecific effects occur. These include widespread cell death (Baat *et al.*, 2001; Lele *et al.*, 2001), defects in epiboly, (Imai and Talbot, 2001), and neural degeneration (Nasevicius and Ekker, 2000; Karlen and Rebagliati, 2001), effects that would not be expected from genetic mutants in which the genes are completely inactivated.

Why morpholinos cause side effects is a major question that remains to be resolved. Clearly, the likely possibilities

are either that there are nonspecific effects of morpholino oligos or contaminants, or that there are effects due to unexpected complementarity of the oligo to other unknown genes. The appearance of these effects is highly oligo-dependent. For example, two groups compared the depletion of *bmp2b* protein using different morpholinos. One oligo, complementary to –61 to –37 of the 5'UTR of *bmp2b* sequence caused the specific dorsalized *swirl* phenotype in 80% of cases at final concentrations of 0.4 μ M, but caused nonspecific epiboly defects and lysis when 1 μ M was injected (Imai and Talbot, 2001). In contrast, a second oligo, designed against –4 to +19, phenocopied the *swirl* mutant at 3.8 μ M, and only caused cell death at doses higher than this (Lele *et al.*, 2001). This difference is not due to variability in calibrating the dose used, since both groups also targeted *bmp7*, this time using the same oligo and having comparable results.

Since it has not been resolved whether side effects are nonspecific or due to the morpholino being complementary to an unknown target, controls for both possibilities are incorporated into most published work (see below).

Oligo Stability

Since morpholino oligos are DNA analogs, they are not susceptible to enzymatic degradation (Hudziak *et al.*, 1996), unlike DNA oligos, and thus have much higher biological stability. This makes it feasible to target genes expressed during organogenesis by injecting oligos into the one- to two-cell-stage embryo. In the case of the *vasa* gene, its protein could not be detected by confocal microscopy 4 days after the initial injection of 12 μ M final concentration (1 ng) of morpholino oligo (Baat *et al.*, 2001). Nasevicius and Ekker (2000), studying the loss of body pigmentation with a *nacre* morpholino, noted nearly complete loss of pigment through 2 days of development. Similarly, morpholino-injected embryos phenocopied the *colourless* mutant in having reduced melanophores after 48 h of development. Here, the oligo-derived mutants were never as complete as the strongest *cls* mutants, since some melanophores were always present (Dutton *et al.*, 2001).

Presumably, oligos eventually lose effectiveness by dilution. Clearly, the length of the loss-of-function effect of an injected oligo depends on the transcription and translation characteristics of the targeted mRNA, as well as the dose of oligo, and will need to be determined for each gene of interest.

How Widely Applicable Are Morpholino Oligos?

Morpholinos have been used in several model organisms in loss-of-function studies. In *Xenopus*, we have routinely used DNA oligos to study maternal gene function, and have, in several cases, compared the effectiveness of morpholinos and DNA oligos. We find there are clear advan-

tages and disadvantages of both approaches. DNA oligos' efficacy can be very easily measured, by comparing the amount of RNA left in control and experimental oocytes after oligo injection, using either PCR or Northern analysis. In comparison, morpholinos do not degrade RNA, and their activity needs to be confirmed in other ways, generally by Western blotting or immunostaining, and antibodies are not always available. Another approach to show their activity is to inject a GFP-tagged version of the mRNA and to show that the oligo reduces GFP fluorescence (Yang *et al.*, 2001). One limitation of this is, however, that the endogenous mRNA and injected version may not have the same accessibility to the morpholino, and so this control does not guarantee that the oligo will have the same effect on the endogenous mRNA.

A clear advantage of morpholinos is that they can be injected directly into fertilized eggs without the toxic effects associated with DNA oligos. For example, when a morpholino oligo against the Wnt pathway component, *β catenin*, was injected at the two-cell stage, it caused complete loss of axial structures, mimicking the effect of a phosphodiester/phosphorothioate oligo injected into the oocyte (Heasman *et al.*, 2000). The effective dose in this case (5–10 ng; 0.6–1.2 μ M final concentration) is very similar to the typical doses used in zebrafish studies. Thus, as long as there is no stored pool of protein, morpholinos can effectively block maternal gene function.

Another perceived advantage of morpholinos is that, since they only work by targeting the start site of the mRNA, it is easier to design an effective oligo. For DNA oligos, five or six oligos are typically tested to find the most efficient one. However, morpholino oligos cannot simply be assumed to be consistently effective. In those cases where more than one oligo has been designed to be complementary to different parts of the 5'UTR sequence of a target mRNA, the oligos have had different degrees of effectiveness in blocking translation (Imai and Talbot, 2001; Lele *et al.*, 2001). This suggests that, as for DNA oligos, we are ignorant of many of the variables underlying morpholino activity.

As in zebrafish studies, it is clear that morpholino oligos may cause side effects in *Xenopus*, and that as the dose increases, the possibility of nonspecific abnormalities also rises. Thus, it is not possible to rely solely upon a dose response for revealing a specific phenotype (Audic *et al.*, 2001). Abnormalities include anterior/posterior truncations, microcephaly, (Nutt *et al.*, 2001), slowed cleavage, delayed development, and widespread cell death (unpublished observations). In some cases, the toxic dose can be clearly distinguished from the effective dose. Nutt *et al.* (2001) found that 1 ng (1.2 μ M final concentration) of morpholino against GFP caused complete inhibition of GFP fluorescence in transgenic embryos carrying an integration of the CMV-GFP transgene, while 40 ng (4.8 μ M final concentration) was injected to cause 45% of the embryos to develop abnormally. In other cases, specific and nonspecific

effects can only be distinguished by the use of appropriate controls.

One type of control frequently used both in *Xenopus* and zebrafish studies is to inject other morpholino oligos, either a nonsense morpholino oligo, an invert of the antisense oligo or a mismatched oligo. These cannot control for the possibility that the experimental oligo is binding to an unexpected mRNA, since they do not have the same sequence. An even worse control is to use the sense oligo, since this should clearly bind to the antisense oligo and block its activity, whether or not that activity is specific. Thus, these types of controls only control for nonspecific toxic contaminants (if they were synthesized at the same time and with the same reagents, machine, and personnel as the experimental oligo).

For DNA antisense oligos, that act by degrading mRNA, specificity can be tested directly, by injecting synthetic mRNA into oocytes after the endogenous mRNA has been degraded by the oligo, and testing its ability to rescue the phenotype. A similar control is necessary for morpholino experiments. However, if the injected mRNA has the 5' sequence complementary to the oligo, it will bind to the oligo and prevent its activity directly, by competing for binding to the endogenous mRNA. This is not a good control for specificity. A better control is to use an mRNA that either lacks the 5' UTR recognized by the oligo (Heasman *et al.*, 2000; Nasevicius and Ekker 2000; Bauer *et al.*, 2001; Satou *et al.*, 2001), or has third base modifications, so that it is no longer recognized by the oligo (Cui *et al.*, 2001). Unfortunately, rescue experiments are often plagued themselves with complications due to the incorrect distribution and activity of the injected mRNA (Sumanas *et al.*, 2001). An alternative approach may be to use two different morpholino oligos to target the mRNA (Howard *et al.*, 2001; Sumanas *et al.*, 2001), and ask if they cause the same phenotype.

Another consideration specific to *X. laevis* is that its allotetraploidy means there are often two orthologs of the gene of interest. These are generally very similar in the coding sequence, but have significant differences in the 5'UTR. So, to target both orthologs, morpholinos have to be designed either to complement the conserved coding region at the start site, or two oligos need to be made (Sumanas and Ekker, 2001). The related frog, *Xenopus tropicalis*, has a diploid genome, and is also amenable to morpholinos (Nutt *et al.*, 2001) and thus may become a more popular model than *X. laevis* for morpholino studies.

Morpholinos have also been tested in ascidian (Satou *et al.*, 2001) and mouse oocytes (Coonrod *et al.*, 2001), and shown to phenocopy the expected phenotypes for loss-of-function of the *β catenin* and *mos* genes, respectively. In both sea urchin and chick embryos, morpholinos have been used to study novel gene function, and in each case, several lines of evidence, as well as the morpholino data were presented to confirm the conclusions (Howard *et al.*, 2001; Kos *et al.*, 2001). Thus, for organisms that have typically lagged behind the genetic model organisms in loss-of-

function assays, morpholinos offer a welcome additional approach.

CONCLUSIONS

In recent months, research on morpholinos has moved beyond the testing phase to the study of novel gene function. Because morpholinos do cause variable side effects and do have variable penetrance, embryos injected with morpholinos may have phenotypes that are difficult to interpret. The loss-of-function effects of morpholinos have to be supported by control mRNA rescue experiments, or, if those experiments are inconclusive, (e.g., because the injected mRNA has pleiotropic effects), by evidence that two morpholinos complementary to different parts of the 5'UTR have the same effect, or by evidence from other overexpression and/or underexpression studies (Ross *et al.*, 2001; Shepherd *et al.*, 2001). In a recent study, for example, two morpholino oligos directed against the zebrafish type 1 serine/threonine kinase receptor *Alk8*, caused a dorsalized phenotype. This was partially rescued by an injected mRNA lacking the 5'UTR sequence targeted by the morpholinos. The authors went on to show that *Alk8* is disrupted in partially dorsalized *lost-a-fin* mutants, and that the mutant is also rescued by *Alk8* mRNA, but not by mRNA for *BMPs2 b* or 7 (Bauer *et al.*, 2001). Together, the data prove the requirement for *Alk8* in the BMP-signaling pathway in zebrafish.

A second example of the use of morpholinos is described by Deardorff *et al.* (2001). A morpholino directed against a *Xenopus* Wnt receptor *Xfz3* was used to test the role of *Xfz3* in neural crest formation. The effectiveness of the oligo in blocking translation was shown by Western blotting with an *Xfz3*-specific antibody. Its ability to block signal transduction was tested in explant assays, by comparing the up-regulation of *Xslug* by *Xwnt1*, in the presence or absence of the MO. The effect of the morpholino oligo was then tested in whole embryos by injecting the oligo into cells fated to form neural crest, i.e., dorsal animal cells at the 32-cell stage. The oligo inhibited neural crest formation and this was rescued by coinjection with mouse *Xfz3* mRNA (Deardorff *et al.*, 2001).

In both these cases, the phenotype seen with the morpholino oligo was supported by other approaches, and there were clear expectations that guided the analysis. Far more challenging will be the use of morpholinos to study completely novel gene function, where there are no clues to distinguish real from nonspecific phenotypes. It will be necessary to have the complete open reading frame of the targeted gene in order to carry out rescue experiments.

In summary, morpholinos have had substantial testing in embryos. As with most injection experiments, they may cause side effects, and may cause an array of phenotypes that require careful interpretation. These drawbacks can be overcome by a variety of controls and by comparison with other loss- and gain-of-function results. The most impor-

tant technical question to answer in their development is why higher doses of biologically inert morpholinos should have side effects. But, with cautious use and clear controls, morpholinos will certainly speed the flow of developmental gene function data.

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Morpholino antisense oligonucleotide induced dystrophin exon 23 skipping in *mdx* mouse muscle

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The *mdx* mouse model of muscular dystrophy arose due to a nonsense mutation in exon 23 of the dystrophin gene. We have previously demonstrated that 2'-O-methyl phosphorothioate antisense oligonucleotides (AOs) can induce removal of exon 23 during processing of the primary transcript. This results in an in-frame mRNA transcript and subsequent expression of a slightly shorter dystrophin protein in *mdx* muscle. Refinement of AO design has allowed efficient exon skipping to be induced in *mdx* mouse muscle cultures at nanomolar concentrations. In contrast, splicing intervention by morpholino AOs has been applied to the β -globin gene pre-mRNA in cultured cells to correct aberrant splicing when delivered in the micromolar range. The morpholino chemistry produces a neutral molecule that has exceptional biological stability but poor cellular delivery. We present data showing that exon skipping in *mdx* cells may be induced by morpholino AOs at nanomolar concentrations when annealed to a sense oligonucleotide or 'leash', and delivered as a cationic lipoplex. We have investigated a number of leash designs and chemistries, including mixed backbone oligonucleotides, and their ability to influence delivery and efficacy of the morpholino AO. Significantly, we detected dystrophin protein synthesis and correct sarcolemmal localisation after intramuscular injection of morpholino AO:leash lipoplexes in *mdx* muscle *in vivo*. We show enhanced delivery of a morpholino AO, enabling the advantageous properties to be exploited for potentially therapeutic outcomes.

INTRODUCTION

The degenerative muscle diseases, Duchenne and Becker muscular dystrophy (DMD/BMD) are allelic X-linked recessive disorders, arising from mutations within the dystrophin gene (1). DMD is characterized by the progressive wasting of skeletal and cardiac muscle and presents between the ages of 3 and 5 years (2). Affected boys are restricted to a wheelchair by the age of 12 years and 90% die in their late teens or early twenties due to cardiac or respiratory failure (2). In the absence of prenatal screening and genetic counselling, DMD occurs at a frequency of ~1 in 3500 live male births (3). DMD typically arises from nonsense or frame-shift mutations within the gene, which cause premature termination of translation and synthesis of a non-functional dystrophin protein. BMD, a milder, allelic form of DMD, manifests as a wide spectrum of phenotypes, from mild to borderline DMD (4). BMD mutations are generally such that the reading-frame

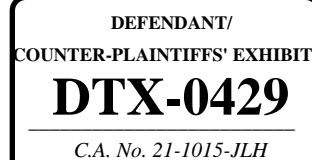
is maintained, enabling a shortened, but still functional, protein to be produced.

Current clinical treatments of DMD/BMD generally involve the use of palliative agents, steroid treatment and intermittent positive pressure ventilation later in life (5,6). However these treatments do not address the primary genetic defect. Several potential therapies have been proposed, including viral-mediated gene transfer, myoblast transplantation, homologous gene up-regulation and targeted gene correction amongst others (7). Although promising, clinical trials have been limited and many hurdles need to be overcome to improve specificity, safety and efficiency.

As an alternative to these potential therapies, we and others have investigated the possibility of using antisense oligonucleotides (AOs) to redirect gene transcript processing and restore dystrophin protein synthesis (8–11). We have previously reported the use of 2'-O-methyl (2OMe) phosphorothioate (PS) AOs to block motifs involved in normal dystrophin pre-mRNA

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splicing in order to induce excision of exon 23, the exon containing the *mdx* nonsense mutation, from the processed mRNA transcript (8–10).

One chemistry that is gaining wide recognition for use in antisense applications is the morpholino oligonucleotide developed by Summerton and Weller (12). These authors developed the morpholino structural type with the intention that this chemistry could provide several advantages in the clinical application(s) of antisense therapeutics, such as strong nucleic acid binding, resistance to nucleases, minimal non-antisense effects, high aqueous solubility and relatively low synthesis costs (12). However, transfection with uncomplexed morpholino AOs (naked transfection) has been shown to require relatively high concentrations (13,14). Because morpholino AOs are neutral molecules, they do not readily diffuse across the cell membrane and cannot be transfected using standard cationic transfection reagents. To overcome this limitation, a morpholino AO directed at the mouse dystrophin exon 23 donor splice site was annealed to various complementary DNA/RNA molecules, or 'leashes' (15).

Varying the length, chemistry and structure of the leash was shown to influence the ability of the morpholino AO to induce exon 23 skipping, presumably by affecting the delivery of the morpholino AO:leash lipoplex to the nucleus. This is the first reported use of a morpholino AO able to efficiently induce the removal of exon 23 from *mdx* dystrophin pre-mRNA as determined by both RT-PCR and western blotting. When delivered as a leash duplex and complexed with Lipofectin, the morpholino AO was effective at concentrations several orders of magnitude lower than previously reported for other applications of this chemistry (13,14). Finally, we also demonstrated dystrophin synthesis and correct sarcolemmal localization *in vivo* in *mdx* mice treated by intramuscular injection of morpholino AO:leash lipoplexes. Our results suggest that the morpholino structural type may be the chemistry of choice for inducing targeted removal of dystrophin exons.

RESULTS

Leash design

We wished to investigate the potential of a morpholino AO to induce dystrophin exon 23 skipping when targeted to the donor splice site of exon 23 (Fig. 1A). Exon skipping is dependent upon the ability of the AO to reach the nucleus and then interfere with spliceosome assembly. To facilitate delivery and nuclear uptake of the morpholino AO, it was annealed to a range of complementary DNA/RNA oligonucleotides, or leashes. It was anticipated from the work of others (15,16), that parameters such as leash annealing length, extent and position of non-complementary overhangs and chemistry could alter the uptake and subsequent biological activity of the morpholino AO. To ensure the leash annealed to the morpholino AO at 37°C, the complementary region consisted of at least 17 bases (15). Various chemistries were also evaluated in leash design to achieve a balance between resistance and susceptibility to degradation (Fig. 1B). It was considered necessary to create a leash sufficiently stable to

ensure delivery to the nucleus while retaining some lability to nucleases to facilitate liberation of the morpholino AO from the AO:leash duplex, and thus enable subsequent annealing to the target sequence. Three chemistries were used with the following hierarchy of nuclease resistance: phosphodiester (PO) < phosphorothioate (PS) < 2OMe PS. Leash arrangements with respect to chemistry, annealing domain and non-complementary overhangs are detailed in Figure 1B. It has been shown that the base composition of the non-complementary overhangs had no significant impact whereas the position of the overhang was more important (15).

Delivery of morpholino:leash duplexes

Prior to transfection with the various morpholino:leash complexes, it was necessary to confirm that all leashes had correctly annealed to the morpholino AO. Morpholino:leash duplexes were electrophoresed through non-denaturing 20% polyacrylamide gels alongside the leash alone to demonstrate a mobility shift, where duplexes were retarded compared to the single-stranded leashes. All leashes were found to have annealed to the morpholino AO (data not shown).

Delivery of a morpholino:leash duplex into the nucleus of the cells was monitored using the morpholino AO annealed to leash 4, which was labelled with FITC-at the 3' end. Untreated cells displayed no fluorescence, whereas cells transfected with ψ M23D(+07–18):leash 4 lipoplexes showed efficient uptake of the complex into the nucleus after 24 h (Fig. 2). While it was possible that intracellular fluorescence was due to transfection of leash 4 only and not the morpholino:leash duplex, several experiments were included to confirm morpholino AO delivery to the nucleus. Firstly, the mobility shift studies indicated that the ψ M23D(+07–18) effectively formed a duplex with leash 4. Cells transfected with the ψ M23D(+07–18):leash 4 lipoplex produced dystrophin gene transcripts missing exon 23 (Fig. 3). Although *H-2K mdx* cells transfected with lipoplexes of leash 4 alone demonstrated identical intracellular fluorescence to cells transfected with ψ M23D(+07–18):leash 4 lipoplex, no transcripts excluding exon 23 were ever observed in cells transfected with individual leashes alone, including leash 4 (data not shown). No intracellular fluorescence was observed when the ψ M23D(+07–18):leash 4 duplexes were transfected in the absence of Lipofectin (uncomplexed), or when the cells were exposed to the supplied FITC-labelled control morpholino, even at high doses of 1 and 10 μ M, in the presence or absence of Lipofectin. Similarly, no exon 23 skipping was observed after transfection of uncomplexed ψ M23D(+07–18):leash duplexes or when ψ M23D(+07–18) was transfected without a leash (Fig. 3). In summary, these results suggest that leashes and Lipofectin were essential for efficient morpholino AO delivery and activity, but did not induce exon 23 skipping on their own.

Assessment of exon skipping induced by morpholino:leash duplexes

Initial experiments designed to assess the ability of morpholino:leash duplexes to induce exon 23 skipping involved transfecting *H-2K mdx* cells, 24 h after seeding, with ψ M23D(+07–18) at 300 nM in combination with each of 12

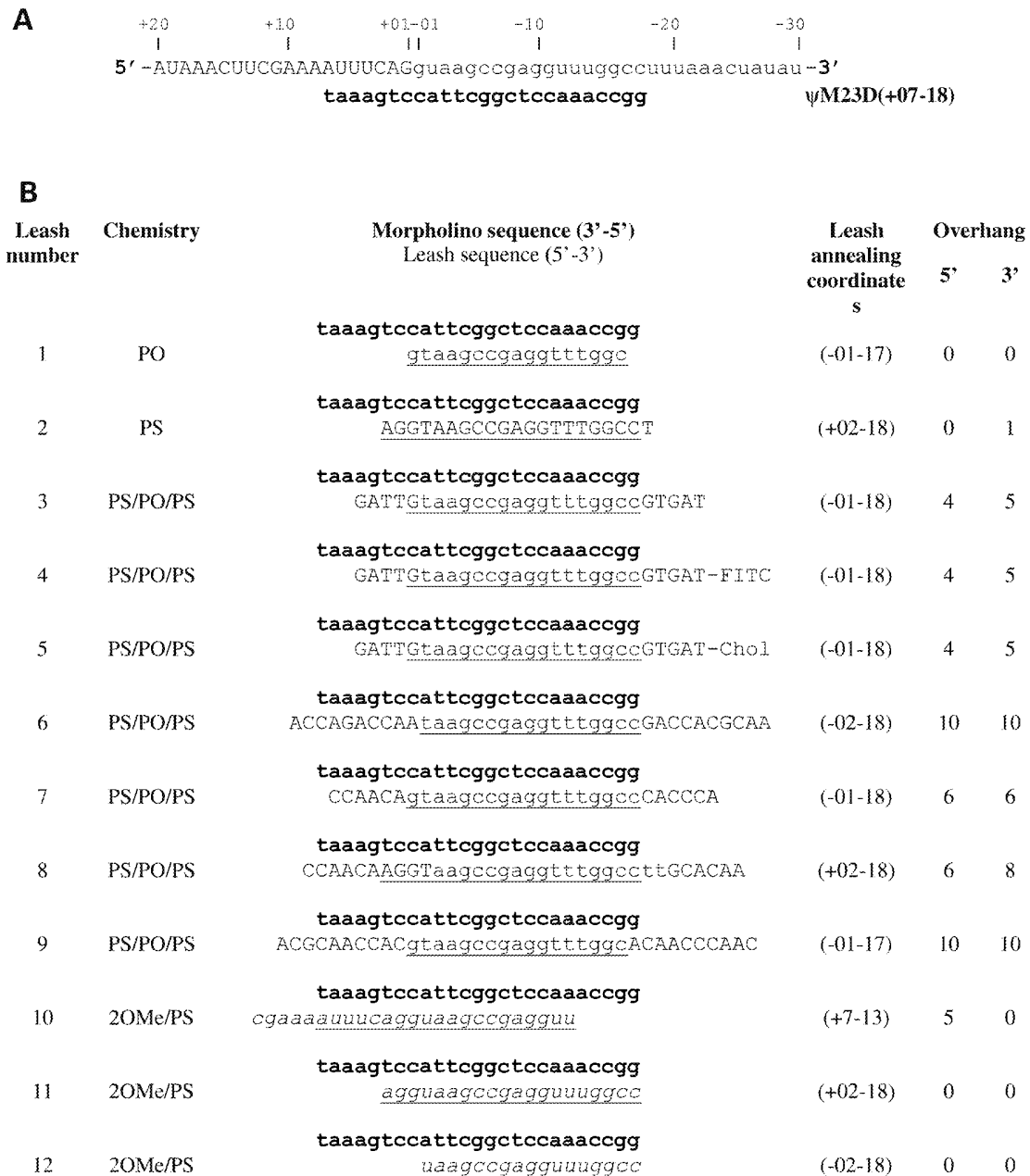


Figure 1. (A) Annealing of the morpholino AO, ψ M23D(+07-18), to the 5' donor splice site of intron 23 in mouse dystrophin pre-mRNA. Exonic region is shown in upper case whereas the intron is shown in lower case. (B) Leashes are shown 5'→3' with annealing positions relative to the morpholino AO ψ M23D(+07-18). Only complementary bases of the leash to the morpholino are underlined. Chemistries are indicated according to the following key: morpholino = bold; phosphodiester = lower case; phosphorothioate = upper case; 2'-O-methyl phosphorothioate = lower case and italics.

different leashes. The nature of the leash was found to influence the ability of the morpholino AO to induce exon skipping, as determined by the presence and relative intensity of the shortened 688 bp RT-PCR product, representing mRNA transcripts missing exon 23, compared with the intact full-length product of 901 bp (Fig. 3).

When transfected as lipoplexes, all 12 ψ M23D(+07-18):leash duplexes demonstrated some capacity to induce exon 23

skipping (Fig. 3). When the morpholino AO was annealed to leash 1, a 17mer complementary PO oligonucleotide, exon 23 skipping was routinely weaker when the morpholino was annealed to a 21mer all PS oligonucleotide (leash 2) with a 20-base complementary core and a single base overhang. Efficient removal of exon 23 from the dystrophin transcript was also observed when the morpholino was annealed to leashes of mixed backbone chemistries (leashes 3-9; Fig. 3). The

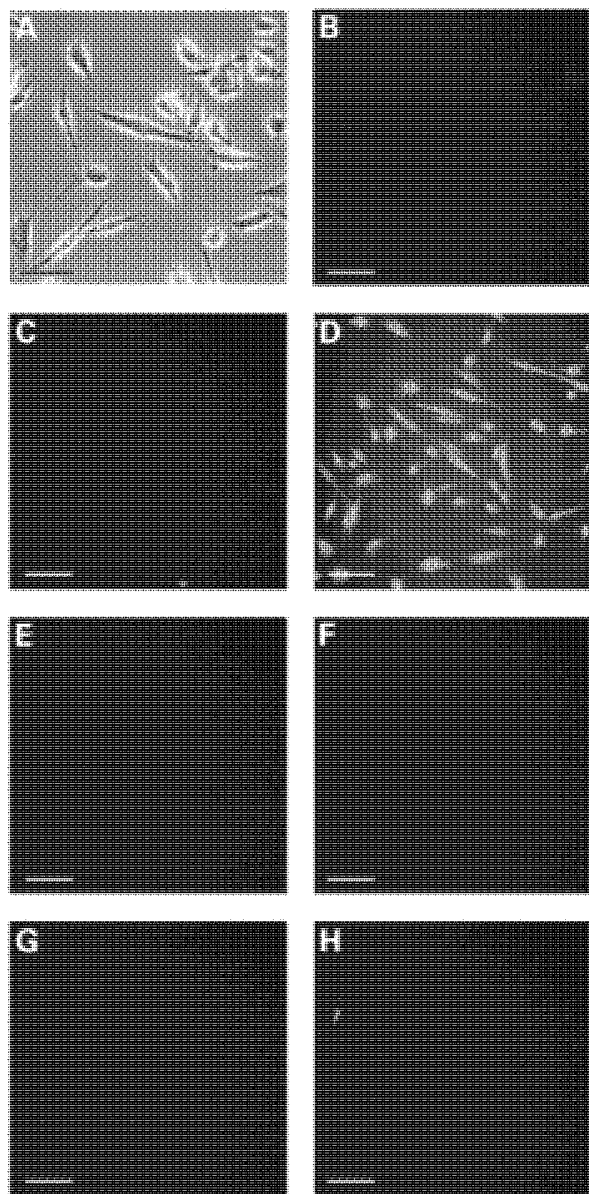


Figure 2. The morpholino AO induced exon skipping in *H-2K mdx* cells only when annealed to a leash, complexed with Lipofectin and transfected as a lipoplex. All doses of ψ M23D(+07-18) were 300 nM unless otherwise indicated. Photographs were taken 24 h after initial exposure to Lipoplexes. (A and B) Untreated cells photographed in phase contrast and with fluorescence excitation respectively. (C) ψ M23D(+07-18):leash 4 uncomplexed (D) ψ M23D(+07-18):leash 4 complexed with Lipofectin. (E) FITC-labelled control morpholino uncomplexed. (F) FITC-labelled control morpholino mixed with Lipofectin. (G) ψ M23D(+07-18):leash 4 uncomplexed at 1 μ M dose. (H) ψ M23D(+07-18):leash 4 uncomplexed at 10 μ M dose. Scale bars = 50 μ m.

simultaneous skipping of exons 22+23 was often observed to varying degrees (Fig. 3). When annealed to leashes of pure 2OMe PS chemistry (leashes 10-12), the induced exon 23 skipping in cells was not as efficient when the shorter leashes (11 and 12) were used. The efficiency of exon skipping was generally found to decrease when the morpholino AO was

annealed to progressively shorter leashes without overhangs (Fig. 1B), as judged by the relative intensity of the 688 bp product to the full length fragment (Fig. 3). No exon 23 or exon 22+23 skipping was ever observed in untreated cells, or those treated with a 300 nM dose of ψ M23D(+07-18) transfected without leash or Lipofectin, or when ψ M23D(+07-18) was annealed to a leash and delivered without Lipofectin.

Titration of ψ M23D(+07-18):leash hybrids

Since all morpholino:leash lipoplexes consistently induced the removal of exon 23 in cultured *H-2K mdx* cells at a transfection dose of 300 nM, titration studies were then performed to determine the minimum effective doses and thus identify the more effective leash designs. To encompass the spectrum of leash designs, six leashes were chosen for titration studies on the basis of their design and chemistry and to refine observed trends. Leashes 1, 2 and 10 were chosen to represent pure PO, PS and 2OMe PS chemistries, respectively, whereas, leashes 3, 6 and 8 represented different facets of the mixed backbone design. The morpholino AO annealed to leashes 3, 6 and 8 by 17-20 complementary PO core bases (Fig. 1B). Leashes 3, 6 and 8 also differed in the number of non-complementary PS bases with 5' and 3' overhangs of 4-10 bases. The six ψ M23D(+07-18):leash duplexes were transfected at doses between 5 and 300 nM with a 2:1 Lipofectin:leash ratio. Morpholino:leash duplexes were also transfected at 300 nM in the absence of Lipofectin.

When the morpholino AO was annealed to leash 1, weak exon 23 skipping was only induced at doses above 100 nM (Fig. 4). Lipoplexes of ψ M23D(+07-18):leash 2 consistently induced strong exon 23 skipping at a transfection dose as low as 30 nM, with weaker, inconsistent skipping evident at 5 nM. Consistent exon skipping was observed when the morpholino AO was annealed to leash 6, compared with the stronger skipping induced by the morpholino AO when it was annealed to leashes 3 or 8 (Figs 3 and 4). The removal of exon 23 from dystrophin mRNA was more prominent at 10 nM when the morpholino was annealed to leash 8, than when it was annealed to leash 3 (Fig. 4). When annealed to leash 10, ψ M23D(+07-18) consistently induced strong skipping at a dose of 30 nM, with weak and inconsistent skipping observed at 10 nM. The induction of exon 22+23 skipping was generally only observed at the higher doses as previously reported for 2OMe PS AOs (10). No exon 23 skipping was observed when duplexes were exposed to cells without prior complexing with Lipofectin, as described above. Furthermore, the minimum effective doses, varying from 5 to 30 nM for the more efficient morpholino:leash lipoplexes, were several orders of magnitude less than the uncomplexed morpholino doses reported as effective by others (13-15). In some instances, weak and inconsistent exon 21 skipping was observed in treated and untreated samples, for example, in the control lanes of the leash 2 experiment (Fig. 4F). This has been reported previously (9,10) and is believed to be a natural event occurring at low levels, and is thus unrelated to the activity of the morpholino AO or leashes.

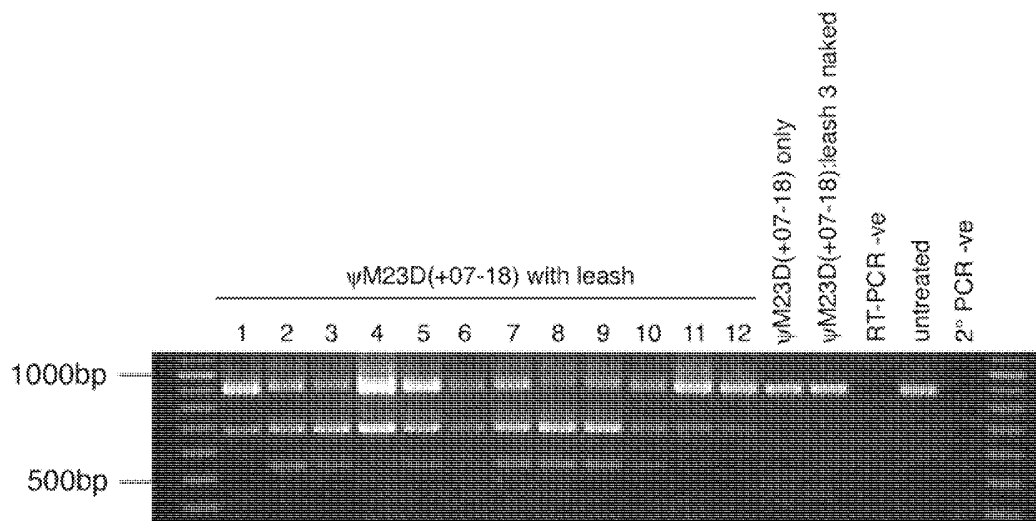


Figure 3. Exon 23 skipping induced by ψ M23D(+07-18) when annealed to different leashes. Twelve leashes were annealed to ψ M23D(+07-18), complexed at a 2:1 (w:w) Lipofectin:leash ratio and delivered to cells at a dose of 300 nM. Total RNA was extracted from cells 24 h after transfection and amplified by nested RT-PCR between exons 20–26. The full-length product of 901 bp was amplified in all samples except PCR-negative controls. A 688 bp product, representing exon 23 exclusion, was detected in samples transfected with morpholino AO:leash duplexes, only after transfection as Lipoplexes. No detectable exon 23 skipping was induced by ψ M23D(+07-18) when delivered without a leash and/or Lipofectin. In addition to exon 23 skipping, a 542 bp product corresponding to exon 22+23 skipping was detected in some samples, as described previously (9,10).

Time course analysis of induced exon skipping

The results of the titration studies yielded several leash designs that were effective at delivering the morpholino AO at low doses. Persistence studies were performed with three leashes, 3, 8 and 10, over a 10 day period to further assess their influence on morpholino AO delivery and the subsequent duration of ψ M23D(+07-18)-induced exon 23 skipping. Cells were transfected with duplexes of ψ M23D(+07-18) and leashes 3, 8 or 10 at 300 and 100 nM doses, 24 h after seeding. Total RNA was extracted 1, 4, 7 and 10 days after transfection and RT-PCR undertaken between exons 20 and 26.

All three morpholino:leash complexes included in this study demonstrated persistent exon 23 skipping for up to 10 days after a single transfection at a dose of 300 nM (Fig. 5). The influence of the leashes on morpholino AO activity was more evident when the morpholino:leash complexes were transfected at 100 nM. At this dose, the morpholino AO induced the most efficient and persistent removal of exon 23 when annealed to leash 3. Exon 23 skipping was consistently shown to be induced efficiently for up to 7 days post transfection, with weaker skipping evident until day 10, the latest time point tested (Fig. 5). The intensity of the 688 bp RT-PCR product, missing exon 23, was strong only at day 1 post-transfection when cells were treated with ψ M23D(+07-18) annealed to leashes 8 or 10. For these treatments, the intensity of the 688 bp band declined after the day 1 time point when compared with the intensity of exon 23 skipping induced by ψ M23D(+07-18):leash 3 duplexes. The persistence of the out-of-frame product missing exons 22+23 was profoundly weaker at the 100 nM dose in all treated samples (Fig. 5). No exon 23 or 22+23 skipping was detected in untreated

cells or cells exposed to naked morpholino:leash duplexes (data not shown).

Western blot analysis of treated cells

We wished to determine if the exon 23 skipping induced by the morpholino AO:leash lipoplexes correlated with the restoration of protein synthesis using the three leashes and a range of control conditions, including transfection with the 2OMe PS AO M23D(+02-18) as a positive control (10). Total protein extracted from treated and control cultures was loaded onto denaturing SDS gradient gels after normalization for the myosin heavy chain, fractionated, transferred to nitrocellulose and detected with the Dys2 monoclonal antibody to the C-terminal of dystrophin (10). Cells were transfected at day 4 in the absence of serum and harvested at day 8.

We detected near-full-length dystrophin in extracts of cells treated with the morpholino AO annealed to leashes 3, 8 and 10 when complexed with Lipofectin (Fig. 6A). Other leashes were not examined, although it was anticipated from RT-PCR studies that variable levels of dystrophin protein would have been produced. Levels of protein induced by two lipoplexes were higher than those achieved after transfection with an equivalent dose of M23D(+02-18), previously the most effective 2OMeAO (10). Levels of dystrophin induced by ψ M23D(+07-18):leash 10 lipoplex were similar to those obtained by treatment with M23D(+02-18) (Fig. 6A), consistent with the slightly weaker influence of leash 10 as determined by RT-PCR analysis (Fig. 5). No protein was observed when *H-2K mdx* cells were exposed to unannealed ψ M23D(+07-18), with or without Lipofectin at 300 nM, or

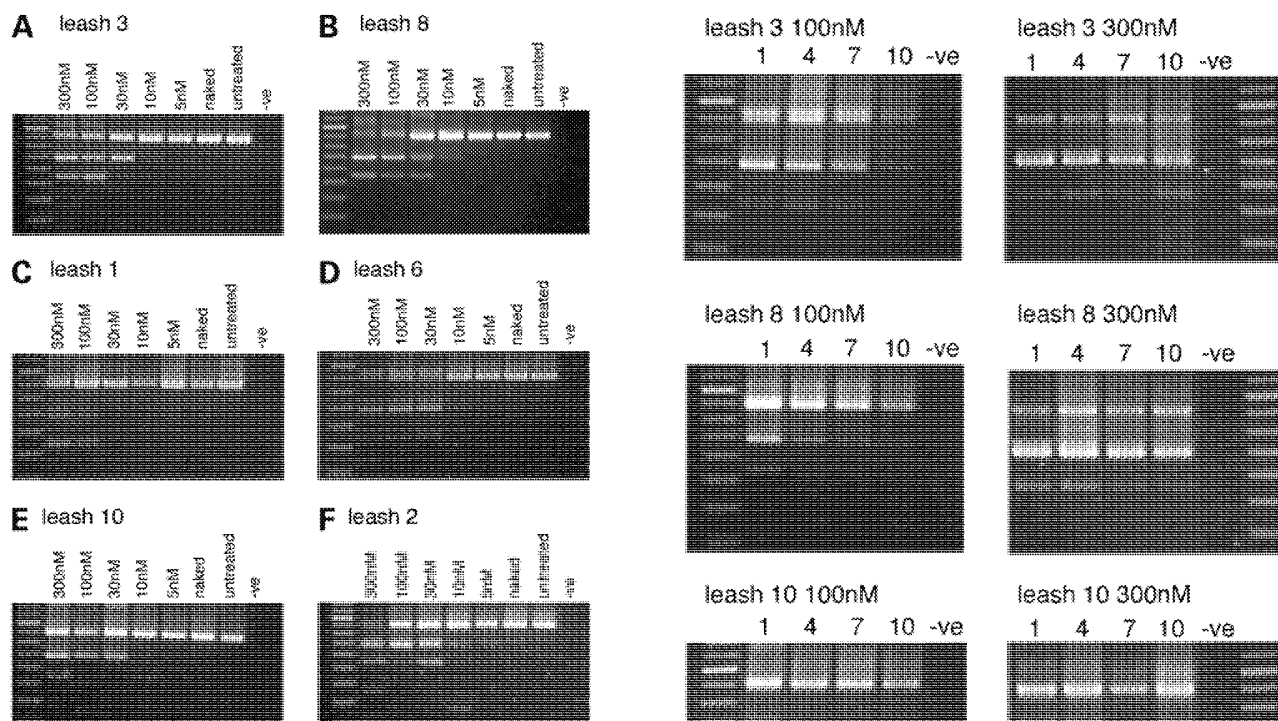


Figure 4. Titration of morpholino:leash duplexes to determine the minimum effective lipoplex dose in tissue culture. ψ M23D(+07-18):leash hybrids were transfected either naked or as lipoplexes at a range of doses. RT-PCR across exons 20-26 was used to ascertain the minimum dose at which each morpholino:leash lipoplex could induce exon 23 skipping (688 bp product). Exon 23 skipping was routinely detected at a minimum dose of 5 nM with leash 2, 10 nM with leashes 3, 6 and 8, 30 nM with leash 10 and 100 nM with leash 1. No exon 23 skipping was detected after naked transfections or in untreated cells. Some sporadic exon skipping was seen in some samples.

when morpholino:leash duplexes were exposed to cells without prior complexing with Lipofectin (Fig. 6A). However, when cells were exposed to a 10 μ M dose of uncomplexed ψ M23D(+07-18) in the absence of serum, low levels of dystrophin were detected upon over-exposure of the membrane (Fig. 6B). In contrast, a single 1 μ M dose administered under identical conditions failed to produce any detectable dystrophin, even after overexposure of the membrane (Fig. 6B). No dystrophin was detected in extracts of cells left untreated or exposed to Lipofectin only [Lipofectin-only dose was equivalent to that administered with ψ M23D(+07-18):leash 3 duplex].

Restoration of dystrophin synthesis *in vivo*

Based on the strength of the *in vitro* results, we injected 1 μ g of ψ M23D(+07-18):leash 3 duplex complexed to Lipofectin at a 2:1 ratio into the left tibialis anterior (TA) muscle of 3-week-old *mdx* mice. The contralateral leg was injected with phosphate buffered saline as an untreated control. Mice were sacrificed 2 days or 2 weeks after injection. Dystrophin was detected and found to be localized to the sarcolemma of muscle fibres in the left TA 2 weeks after injection (Fig. 7C and D). The dystrophin signal was strong and continuous, reminiscent

Figure 5. Time course analysis of exon 23 skipping induced by morpholino:leash complexes. *H-2K mdx* cells were transfected 24 h after seeding with either 100 or 300 nM doses of ψ M23D(+07-18):leash lipoplex. Total RNA was extracted 1, 4, 7 or 10 days after transfection for RT-PCR across exons 20-26. The 688 bp product representing exon 23 skipping was detected for up to 10 days with all leashes and doses although at different intensities relative to the intact product. As expected, skipping was more pronounced with all morpholino:leash lipoplexes at 300 nM than at 100 nM. The most efficient skipping at 100 nM was observed with leash 3. At 100 nM, all other morpholino AO:leash lipoplexes produced comparable levels of exon 23 skipping that were sustained for 10 days, but at lower levels than those produced by the ψ M23D(+07-18):leash 3 complex.

of the C57Bl/10ScSn normal control (Fig. 7A). The induced protein was determined to be near full-length in size by western blotting (not shown). Dystrophin was not detected in the TA from the contralateral, untreated limb (Fig. 7B), examined after sham injection. Sections of treated muscle examined 2 days after injection only showed low levels of diffuse staining for dystrophin (data not shown). A 688 bp product representing the dystrophin transcript missing exon 23 was detected in the treated left TAs 2 days and 2 weeks after injection, but not in

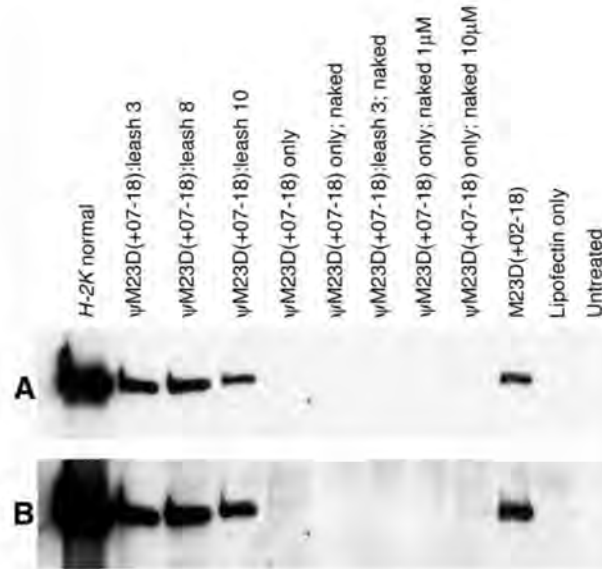


Figure 6. Western blot analysis of dystrophin in treated and control cultures. *H-2K mdx* cells were transfected 4 days after seeding in six-well plates and total protein harvested 4 days later. Samples were loaded after normalization for myosin heavy chain. All cells were transfected at 300 nM with a 2:1 (w:w) ratio of Lipofectin:leash (or AO) unless indicated otherwise. The positive control consisted of untreated *H-2K* normal myogenic cells harvested on day 8 after seeding (upper panel). After overexposure of the membrane, very low levels of dystrophin could be detected in cells after treatment with a single 10 μ M dose of ψ M23D(+07-18) in the absence of serum, but not with the 1 μ M dose (lower panel).

the untreated contralateral TAs (Fig. 7E). The persistence of the induced dystrophin mRNA transcript missing exon 23 for at least 2 weeks is of longer duration than we have observed when using the 2OMe PS AO M23D(+12-13) *in vivo* (9) (unpublished observations). This is likely to be the result of the more stable chemistry and/or perhaps the slow release of the morpholino AO due to the presence of the leash. The identity of the induced product was confirmed by direct DNA sequencing, which also showed that the fidelity of the exon boundaries had been maintained (data not shown).

DISCUSSION

AOs have been successfully used to produce shortened mRNAs by inducing specific exon skipping of dystrophin transcripts in *mdx* muscle *in vitro* and *in vivo*, and in cultured human cells (8,9,11,17,18). Previously, our laboratory has reported that the efficiency of exon 23 skipping induced by 2OMe PS AOs in *mdx* muscle cells was dependant on the target site of the AO rather than the length of the AO (9). Recent improvements to the target design of the 2OMe PS AOs identified a 20mer M23D(+02-18) and a 17mer M23D(-02-18), which induced enhanced levels of exon skipping and protein synthesis (10). When targeting exon 23 for omission from the pre-mRNA, out-of-frame transcripts missing both exons 22 and 23 were frequently detected and reflect the intimate processing of these 2 exons (10). The generation of dystrophin transcripts skipping exons 22 and 23 does not reflect a lack of specificity in the AO

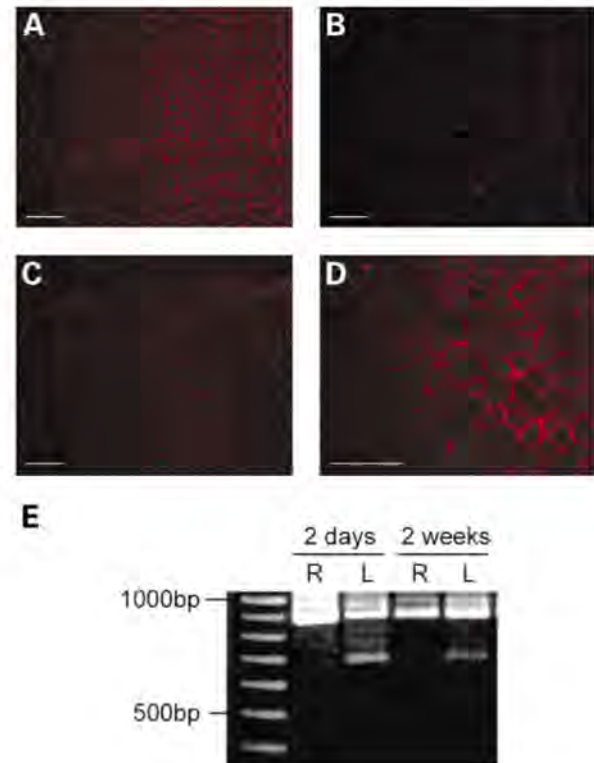


Figure 7. Localization of induced dystrophin protein at the sarcolemma and detection of mRNA missing exon 23 in *mdx* muscle after a single injection of ψ M23D(+07-18):leash 3 lipoplexes. The left TA muscles of *mdx* mice were treated with a single 10 μ l intramuscular injection of 1 μ g of ψ M23D(+07-18) annealed to leash 3 and complexed with Lipofectin at a 2:1 (w:w) Lipofectin:leash ratio. The right leg was sham injected. (A) C57Bl/10ScSn normal muscle immunostained with Dys2. (B) Right sham injected TA of *mdx* muscle demonstrating the absence of dystrophin. (C and D) Two magnifications of the left TA of a treated *mdx* mouse sacrificed 2 weeks after injection, demonstrating the restoration of dystrophin to the sarcolemma. Scale bars = 50 μ m. (E) Detection of the 688 bp RT-PCR product representing the mRNA missing exon 23 in the left TA 2 days and 2 weeks after a single treatment. No exon 23 skipping was detected in the contralateral muscle.

target, that is the donor splice site, but rather the processing of the targeted exon (18). With these principles and improvements further established, investigating alternative oligonucleotide structural types is an important consideration, as it could ultimately yield a molecule with enhanced safety, efficiency, specificity and affordability. The feasibility of using alternative oligonucleotide chemistries to modulate the splicing process has only been explored for the dystrophin pre-mRNA using the nuclease-sensitive PO chemistry (19).

The morpholino structural type was developed in response to the limitations and non-specific effects observed with some early AO molecules (12). The unnatural morpholino chemistry renders the molecule highly resistant to nucleases. An essential property for our application is that the morpholino AOs will not induce RNase H-mediated down-regulation of the target pre-mRNA (20). However, the uncharged backbone compromises delivery, for non-ionic AOs cannot easily be delivered into cultured cells using delivery agents such as cationic liposomes. To circumvent this difficulty, we investigated the use of single stranded (anionic) nucleic acid 'leashes' which were annealed

to the morpholino AO, allowing the AO:leash duplex to be complexed with Lipofectin.

In this study, a single morpholino AO was used, whilst the annealed leashes varied, with several parameters considered significant. Firstly, the length of complementary core sequence defines the melting temperature (T_m) and has been shown to greatly influence the activity of peptide nucleic acid AOs to down-regulate luciferase expression in co-transfected COS-7 cells (16). Secondly, another study used a morpholino AO in an inducible luciferase system (21) and proposed that the presence of non-complementary overhangs, particularly a 5' overhang, could increase luciferase expression by increasing accessibility of nucleases which liberated the morpholino AO from the leash (15). We also investigated the use of mixed chemistry backbones which have previously been used for therapeutic AOs designed to down-regulate gene expression (22), but to our knowledge have never been employed in leash-like molecules. Finally, a cholesterol moiety was included at the 3' end of leash 5 in an attempt to enhance uptake of the morpholino:leash lipoplex.

The pure PO chemistry leash, leash 1, was consistently less effective than the other leashes at delivering the morpholino AO to promote dystrophin exon 23 skipping. This may have been due to the labile PO backbone being too rapidly degraded, or the absence of any non-complementary overhang. Duplexes comprising of morpholino AO:leash 2, the pure PS chemistry leash, were able to induce exon skipping at much lower doses after transfection *in vitro*. This could be due to either the increased leash stability and/or the leash length, which subsequently improved delivery of the morpholino AO to the nucleus.

The proposed requirement for nucleases to degrade the leash and liberate the morpholino AO is not supported by the observation that the more stable 2OMe PS leashes 10, 11 and 12 facilitated exon skipping with a hierarchy of efficiency $10 > 11 > 12$, inversely proportional to their length. The 2OMe PS leashes are several-fold more nuclease resistant than the PO or PS oligonucleotides (23,24). Leash 10 contained a 5 base overhang at the 5' end of the leash and was consistently found to be more effective in delivery of the morpholino AO as determined by relative amounts of induced exon skipping. Indeed, we observed that the morpholino AO was less effective when annealed to pure 2OMe PS leashes without overhangs (leashes 11 and 12; Fig. 3). It must be considered that the leash length and/or the overhang may mediate enhanced lipoplex formation, potentially through the formation of higher order structures that affect complexing with Lipofectin.

Both leash 2 and leash 10 were complementary to ψ M23D(+07-18) over a 20 nucleotide region, although at different annealing sites, suggesting that the T_m s of the two morpholino:leash duplexes should be similar. However, the two leash chemistries have contrasting effects on the T_m where PS modifications decrease the T_m while 2OMe modifications enhance binding to the target (25). Thus, the T_m of leash 10 should be slightly higher than that of leash 2. The use of mixed PO/PS structural designs proved effective in delivering the morpholino AO to the cell nucleus. When the morpholino was annealed to mixed backbone leashes (leashes 3, 6 and 8), exon 23 skipping was consistently shown to be induced at doses as low as 10 nM. In assessing the mixed backbone leashes, the

presence of the non-complementary overhangs also appeared to be an important factor. It is interesting to note a report that leash 5' overhangs increase susceptibility to nucleases (15), despite the accepted convention that most exonuclease degradation of AOs occurs in the 3'-5' direction (26,27). Leashes 4 and 5 were of identical nucleotide composition to leash 3 and differed only by either an FITC or cholesterol group at the 3' terminus, respectively. The addition of either FITC or cholesterol moiety appeared to lower the efficiency of exon skipping after transfection of the morpholino:leash lipoplex when compared with leash 3.

The detection of dystrophin protein induced by the ψ M23D(+07-18):leash 3 lipoplex is one of only a few reports to date describing *in vivo* results achieved with AO molecules designed to induce dystrophin exon skipping and the first using the morpholino chemistry. The TA muscle fibres positively stained by the dystrophin monoclonal antibody are not likely to be revertant fibres, since the latter usually only occur as single fibres or small clusters (28). Furthermore, the protein detected by western blotting was near full-length, and presumed to be missing only the 71 amino acids encoded by exon 23 by extrapolation from the *in vivo* RT-PCR assay. Studies suggest that revertant fibres skip multiple exons at a time (29) and they occur at levels generally too low to be detectable by western blotting (30,31). The weaker diffuse dystrophin immunostaining on sections of muscle treated with the morpholino:leash lipoplex after only 2 days most likely reflects the time required to transcribe and translate the induced exon 23 skipped dystrophin mRNA and the subsequent localization of the dystrophin. The nuclease resistance associated with the morpholino chemistry (20) and the stability of the induced dystrophin mRNA transcript are likely to be a major factors contributing to the continued presence of the induced transcript 2 weeks after treatment *in vivo*.

In some cases, restoration of the dystrophin-associated protein complex has been shown to be insufficient to reverse or prevent the pathological process in muscular dystrophy (32). Therefore, further tests in addition to immunohistochemical localization are needed to demonstrate restored function, although no convention currently exists for either defining the minimum number of tests to prove functionality or to provide a relative measure between groups (33,34). Our purpose was to demonstrate the potential and feasibility of using morpholino AO:leash lipoplexes to induce exon 23 skipping and protein restoration, thus experiments to ascertain function are beyond the scope of this report.

For clinical applications of AOs as a potential therapy for DMD, AO delivery needs to be efficient, tissue specific (if possible) and have negligible side-effects. Our results, coupled with the chemical properties of the morpholino structural type (12), suggest that morpholino AOs may be well suited for therapeutic induction of exon skipping. Furthermore, when annealed to leash 3 or 8, the levels of induced dystrophin protein were higher than those observed after transfection of an equivalent dose of 2OMe PS M23D(+02-18). However, as the target sites of the two AOs differ by five nucleotides, in addition to the chemistries, generalizations regarding the superiority of the morpholino over the 2OMe PS chemistry are purely speculative at this stage. We are expanding this work by directly comparing a range of chemistries at the target sites

to further explore the relationship of chemistry and target site sensitivity (B.L. Gebiski, S. Fletcher and S.D. Wilton, manuscript in preparation).

Morpholino AOs have been previously reported to be able to restore correct β -globin splicing in erythroid cells from thalassemic patients (35). However, concentrations as high as 45 μ M were required when the morpholino was delivered by a syringe-loading method to suspension cultures of mononuclear cells (equivalent to scrape loading) (35). Similarly, high doses of morpholino were required over longer periods in order to produce the same effect after free uptake of morpholino AO in a related cell line (14). Delivery of morpholino AOs into cultured HeLa cells, when annealed to a DNA leash and complexed with the weakly basic molecule ethoxylated polyethylenimine (EPEI), resulted in improved activity (15). When comparing this delivery system to the conventional scrape loading method at the same dose, 1 μ M of the morpholino:DNA hybrid complexed with EPEI yielded a 10-fold increase in luciferase activity (15). These reports serve to emphasize the benefits of using a leash to enhance delivery of a morpholino AO. Although the use of greatly reduced AO doses may be preferable in a clinical setting, the requirement for delivery reagents contribute their own disadvantages, such as potential toxicity (36,37) and/or modified tissue distribution and potential serum and cell interactions (36). Exploring the use of various chemical structural types and/or modifications to AO design should ultimately lead to compromises where optimal efficiency, stability and safety of AOs and delivery agents can be achieved.

This is the first study in which morpholino AOs have been used to restore gene function in the *mdx* mouse model of DMD, both *in vitro* and *in vivo*. Data presented here has demonstrated that efficient exon 23 skipping and restoration of protein synthesis can be induced by a morpholino AO, ψ M23D(+07–18), when annealed to a range of leashes and delivered as lipoplexes. Exploration of leash design resulted in improved delivery of a morpholino AO, such that effective doses were several orders of magnitude lower than those reported by others (14,15,35).

While leash design will obviously be restricted to complementary regions of the morpholino AO, more important factors appear to be the length of the leash and non-complementary overhangs at one or both termini. It has been reported that the activity associated with the non-complementary overhangs was sequence-independent (15). Since the sequence of the overhangs in this study were different from those used by others, it is most likely that this approach can be readily applied to a wide range of morpholino AOs directed at other gene transcripts. Morpholino AOs thus appear favourable for future *in vivo* studies as they have also been reported to be stable in serum and plasma.

MATERIALS AND METHODS

Morpholino AO design and synthesis

Morpholino AOs were purchased from Gene Tools, USA and were HPLC-purified. Previous studies indicated that the 2OMe AO M23D(+02–18) directed against the 5' donor splice site of

intron 23 was the most efficient AO tested for inducing exon 23 skipping (10). The sequence of this AO was supplied to Gene Tools, who recommended that the morpholino AO be at least 25 bases in length. The morpholino AO was redesigned such that it extended five bases further into exon 23 (Fig. 1A) and had the coordinates M23D(+07–18) according to previously described nomenclature (10). We have used the prefix ' ψ ' throughout to indicate the morpholino chemistry. For fluorescence studies, we also employed the Gene Tools supplied FITC-labelled control morpholino AO (5'-CCTCTTACCTCAGTTACAATTATA-3'; see www.gene-tools.com/Antisense/body_products.HTML#StandardControl). The morpholino AO was resuspended in 600 μ l of distilled water under sterile conditions to produce a stock concentration of 500 μ M and stored at -20°C . Stock solutions were warmed to room temperature, and then pre-heated to 65°C for 5 min before being added to the annealing mixture.

Design and synthesis of oligonucleotide leashes

All leash oligonucleotides were synthesized on an Expedite 8909 (Applied Biosystems) using a modified 1 μ mol thioate synthesis protocol. Leashes 10, 11 and 12 were synthesized as 2OMe PS and HPLC-purified by Geneworks (Adelaide, Australia). All leash sequences, chemistries and annealing positions relative to ψ M23D(+07–18) are indicated in Figure 1B.

Annealing of the morpholino AO to the leashes

The morpholino AO was annealed to the leashes according to the method of Braasch and Corey described for preparing PNA:DNA complexes (38). Briefly, all morpholino:leash working solutions were prepared at a final concentration of 60 μ M. Each solution contained the morpholino and respective leash at a 1:1 (mol:mol) ratio using 6 μ l of 500 μ M morpholino stock solution. The volume of leash added to each mix depended on the individual leash concentration. For all annealing reactions, 12.5 μ l of 10 \times PBS (pH 7.4) was added to a microfuge tube resulting in a final concentration of 2.5 \times PBS in 50 μ l. Tubes were incubated in an MJ research thermal cycler equipped with a hot bonnet according to the following temperature profile: 95°C for 5 min, 85°C for 1 min, 75°C for 1 min, 65°C for 5 min, 55°C for 1 min, 45°C for 1 min, 35°C for 5 min, 25°C for 1 min and 15°C for 1 min. Working stocks were stored at 4°C .

Confirmation of successful annealing of morpholino AO to leashes

To confirm that the leash oligonucleotides had annealed to the morpholino AO, complexed samples were assessed for a mobility shift after non-denaturing polyacrylamide gel electrophoresis. Samples were prepared by mixing 1 μ l of the morpholino:leash complex, 2 μ l of PBS and 1 μ l of water. Samples were then incubated at 37°C for 30 min. Following incubation, 1 μ l of 5 \times glycerol loading buffer was added and samples were electrophoresed through a 19:1 20% polyacrylamide non-denaturing gel in TBE running buffer at 175 V for 100 min. Gels were stained with Sybr Gold (Molecular Probes,

OR, USA) and photographed under UV light trans-illumination using a Kodak ID 2.0 gel documentation system.

Cell culture and transfection

H-2K^b-tsA58 (H-2K) mdx cells were cultured as described previously (9,10). Morpholino:leash duplexes were delivered to the nucleus of the cells with Lipofectin (Invitrogen, Melbourne, Australia) at the ratio of 2:1 Lipofectin:AO (9,10). The amount of Lipofectin used to deliver each morpholino:leash complex was calculated on the w:w ratio of Lipofectin:leash, as it is the charged leash that interacts with Lipofectin. When delivering AOs of the 2OMe PS chemistry using Lipofectin, lipoplexes were formed on the basis of 2:1 (w:w) ratio of Lipofectin:AO as previously described. All lipoplexes were prepared in serum-free OptiMEM (Invitrogen) according to the manufacturer's instructions, to a final transfection volume of 500 µl/well of a 24-well plate. Since morpholino:leash complexes were always formed at a 1:1 mol:mol ratio, the concentration indicated is a measure of each component.

RNA extraction, RT-PCR analysis

RNA was extracted using RNA Bee (Tel-Test, Friendswood, TX, USA) 24 h after transfection as described previously (9). For persistence studies, RNA was extracted 24 h after transfection, then on days 4, 7 and 10. RT-PCR was also performed as described (9).

Protein extraction and western blots

Protein extraction and western blots were performed as described previously (9,10). All *in vitro* samples were loaded on a denaturing 4–8% gradient gel after normalization for myosin heavy chain as described.

In vivo treatment and immunohistochemistry

Twenty-one-day-old C57Bl/10ScSn *mdx* mice were given a single 1 µg dose of ψM23D(+07–18):leash 3 duplex complexed with Lipofectin at a 2:1 (w:w) Lipofectin:leash ratio in a 10 µl volume of phosphate buffered saline. The left tibialis anterior (TA) muscle was injected with the morpholino:leash lipoplex while the right was injected with phosphate buffered saline. Lipoplexes were prepared in saline as previously described (9). Mice were sacrificed either 2 days or 2 weeks after treatment. Injected and contralateral muscles were dissected, embedded in OCT compound (Tissue-Tek, Sakura Finechemicals, Tokyo, Japan) and snap frozen in liquid nitrogen-cooled isopentane and stored at –80°C. Serial 10 µm frozen sections were adhered to silanated slides for immunohistochemistry or collected in tubes for either protein or total RNA studies as described above. Immunohistochemistry was performed using the Mouse-on-Mouse kit with Texas Red substrate according to the manufacturer's instructions (Vector Laboratories, Burlingame, USA) with the monoclonal antibody DYS2 (1:30; Novocastra, Newcastle-upon-Tyne, UK). Fluorescence was visualized with an Olympus IX70 microscope and images recorded with an Olympus DP11 digital camera.

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Exhibit 15



SRPT-VYDS-0002984

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 15, 2017
Electronic Signature for Amy E. Mandragouras, Esq.: /Amy E. Mandragouras, Esq./

Docket No.: AVN-008CN41
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Stephen Donald Wilton *et al.*

Application No.: 15/705,172

Confirmation No.: 2879

Filed: September 14, 2017

Art Unit: 1674

For: ANTISENSE OLIGONUCLEOTIDES FOR
INDUCING EXON SKIPPING AND
METHODS OF USE THEREOF

Examiner: Not Yet Assigned

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

Application No.: 15/705,172

Docket No.: AVN-008CN41

AMENDMENTS TO THE CLAIMS

1. **(Canceled)**
2. **(New)** An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.
3. **(New)** A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

Application No.: 15/705,172

Docket No.: AVN-008CN41

REMARKS

Claim 1 was pending in the application. Claim 1 has been cancelled without disclaimer or prejudice to further prosecution in this or a related application. New claims 2 and 3 have been added.

Support for the new claims can be found throughout the specification and claims as originally filed. Specifically, support for the term "morpholino antisense oligonucleotide" can be found at page 17, lines 1-5 (Table 1A) of the specification. Morpholino antisense oligonucleotides have been described in the literature. See, *e.g.*, Summerton, J. and Weller, D. (1997) Morpholino Antisense oligomers: design, preparation, and properties. *Antisense Nucl. Acid Drug Dev.*, 7, 187-195; Heasman, J. (2002) Morpholino Oligos: making sense of antisense? *Dev Biol* 243:209-214; and Gebiski, B. *et al.* (2003) Morpholino antisense oligonucleotide induced dystrophin exon 23 skipping in *mdx* mouse muscle. *Hum. Mol. Gen.* 12(15): 1801-1811.

No new matter has been added. Accordingly, following entry of the foregoing amendment claims 2 and 23 will be pending in the application.

Application No.: 15/705,172

Docket No.: AVN-008CN41

CONCLUSION

In view of the foregoing, Applicants respectfully submit that the pending claims are in condition for allowance. If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 217-4626. If a fee is due with this submission, please charge our Deposit Account No. 12-0080 under Order No. AVN-008CN41, from which the undersigned is authorized to draw.

Dated: September 15, 2017

Respectfully submitted,
Electronic signature: /Amy E. Mandragouras,
Esq./
Amy E. Mandragouras, Esq.
Registration No.: 36,207
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
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(617) 217-4626
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

Exhibit 16

**IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE**

<hr/>)	
NIPPON SHINYAKU CO., LTD.,)	
Plaintiff,)	
)	
v.)	
)	C.A. No. 21-1015 (GBW)
SAREPTA THERAPEUTICS, INC.,)	
Defendant.)	
<hr/>)	
SAREPTA THERAPEUTICS, INC. and)	
THE UNIVERSITY OF WESTERN)	
AUSTRALIA, Defendant and Counter-)	
Plaintiff)	
)	
v.)	
)	
NIPPON SHINYAKU CO., LTD. and)	
NS PHARMA, INC., Plaintiff and)	
Counter-Defendants.)	
<hr/>		

EXPERT REPORT OF DR. MATTHEW J.A. WOOD

September 8, 2023



Matthew J.A. Wood, F.Med.Sci., MA, D.Phil.

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TABLE OF EXHIBITS

Exhibit 1	List of Materials Reviewed.
Exhibit 2	Curriculum Vitae.

I. INTRODUCTION AND ASSIGNMENT

1. I have been asked by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) to provide an opinion concerning the state of the art of exon skipping therapies for treatment of Duchenne muscular dystrophy (“DMD”) and what a person of ordinary skill in the art (“POSA”) would have understood the inventors of the asserted patents to have invented based on the Specification. This expert report (“Report”) presents the opinions I have formed at this time. If asked, I will testify based upon my study of the materials identified in Exhibit 1 and throughout this Report, as well as my own previous knowledge and experience on this subject matter.

2. I understand that NS alleges that Sarepta Therapeutics, Inc. (“Sarepta”) infringes several of NS’s patents and that Sarepta alleges that NS infringes three patents owned by the University of Western Australia (“UWA”) and exclusively licensed by Sarepta. I have reviewed only publicly available information.

3. I understand from counsel for NS (“counsel”) that I may be provided additional information as this case proceeds, including rebuttal opinions that may be offered by experts for Sarepta Therapeutics, Inc. (“Sarepta”) and the University of Western Australia (“UWA”). Accordingly, I may need to change or augment my analysis and opinions in light of any new information or evidence that is presented after this Report. I expressly reserve the right to do so, including to opine on any evidence raised in those rebuttal opinions.

4. My consulting rate for this case is £650 GBP per hour. My compensation is not related to the outcome of this action, and I have no financial interest in the outcome of this case.

II. QUALIFICATIONS

5. My qualifications to testify about my opinions herein, the UWA Patents, and relevant technology described in my curriculum vitae (“CV”), which is attached as Exhibit 2, and are summarized below. My CV includes my educational background and academic work history.

6. I am a Professor of Neuroscience at the University of Oxford in the United Kingdom. I am also a Professorial Fellow at Somerville College, one of the colleges of the University of Oxford.

7. In 1987, I earned a Bachelor’s degree in Medicine and Surgery at University of Cape Town (Cape Town, South Africa). By 1992, I had completed my D. Phil in Medicine/Physiological Science at University of Oxford (Oxford, UK).

8. From 1994-1998, I was a University Lecturer in Anatomical Science in the Department of Human Anatomy and Genetics at the University of Oxford. From 1999-2010, I was a University Lecturer in Biomedical Science in the Department of Physiology, Anatomy, and Genetics at the University of Oxford. Since 2010 I have been Professor of Neuroscience first in the Department of Physiology, Anatomy, and Genetics and since 2018 in the Department of Paediatrics at the University of Oxford. From 2013-2016, I was the Associate Head of the Medical Sciences Division at the University of Oxford. Since 2016, I have been the Deputy Head of the Medical Sciences Division at the University of Oxford.

9. My laboratory researches gene therapies for degenerative disorders of the nervous system and muscle, including DMD. The main focus of our research is the investigation of novel therapeutic approaches using short nucleic acids that target RNA and in particular the development of novel delivery methodologies to enhance the efficacy of therapeutic nucleic acids including

antisense oligonucleotide agents for DMD. Such methodologies include the development of peptide-based and nanotechnology-based delivery agents.

10. As my CV shows, I have roughly 350 peer-reviewed publications in this field, with many additional extramural invited presentations, oral presentations, and poster presentations.

11. I have received awards and recognition for my work, both internally within my research institution and externally from other organizations, including numerous awarded grants. Of particular note, in 2020 I became an Elected Fellow of the Academy of Medical Sciences in the United Kingdom.

12. My professional affiliations, publications, patents, and honors are further detailed in my CV, attached as Exhibit 2.

III. BACKGROUND OF ANALYSIS

13. In considering and forming my opinions, I have reviewed and analyzed the information and materials identified in this Report. As stated, a list of the materials I reviewed in preparation of this Report is attached as Exhibit 1.

14. I am not an attorney and do not have formal training in the law regarding patents. This section presents my understanding of currently applicable legal principles, explained to me by counsel, which I have used in forming my opinions.

A. Person of Ordinary Skill in the Art

15. I am informed that a person of ordinary skill in the art (“POSA”) is a hypothetical person skilled in the relevant art, not a judge, not a layperson, not a person skilled in the remote arts, and not a genius in the relevant art. Relevant factors in determining the level of ordinary skill in the art include the educational level of the inventors of the patent in suit and of those working in the field at the relevant time. Other relevant considerations include various prior art approaches

employed in the art, types of problems encountered in the art, the rapidity with which innovations are made, and the sophistication of the technology involved.

B. Inventorship

16. I am informed that inventorship occurs when an inventor forms in his mind a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice. An idea is definite and permanent when the inventor has a specific, settled idea, a particular solution to the problem at hand, not just a general goal or research plan he hopes to pursue. I am informed that in an unpredictable field like antisense oligonucleotides for exon skipping, the inventor must contemporaneously recognize and appreciate the invention, and the invention must have known utility for there to be invention.

IV. BACKGROUND REGARDING THE SCOPE AND CONTENT OF THE PRIOR ART AT THE TIME OF THE CLAIMED INVENTION

17. The following is largely drawn from the Technical Background section of my November 28, 2014 declaration submitted to the United States Patent & Trademark Office Patent Trial and Appeal Board in Interference Nos. 106,007, 106,008, and 106,013 in support of UWA's positions in those interferences. Interference No. 106,007 Ex. 2081 at ¶¶ 13-108; Interference No. 106,008 Ex. 2081 at ¶¶ 13-108; Interference No. 106,013 Ex. 2081 at ¶¶ 13-108. Because many aspects of the technical background have not changed since 2014, I have adapted those paragraphs and reiterated them below.

A. Duchenne Muscular Dystrophy

18. DMD is an X-chromosome-linked neuromuscular genetic disease that occurs in approximately one in every 3,500 boys born worldwide. It primarily affects male children, with most boys diagnosed between the ages of 3 to 5 years.

19. Symptoms of DMD usually appear in infants and toddlers. Affected children may experience developmental delays such as difficulty in walking, climbing stairs or standing from a sitting position. As the disease progresses, muscle weakness in the lower limbs spreads to the arms, neck and other areas. Most patients require full-time use of a wheelchair in their early teens, and then progressively lose the ability to independently perform activities of daily living such as using the restroom, bathing, and feeding.

20. Upon further progression of the disease, patients experience increased difficulty in breathing due to respiratory muscle dysfunction and typically require ventilation support. Additionally, cardiac dysfunction can lead to heart failure. DMD is universally fatal, with patients usually succumbing to the disease in their twenties.

21. DMD is caused by a change(s) (known as a mutation) in the gene that encodes a protein called dystrophin. These mutations may include large deletions (about 60-70 percent), large duplications (about 10 percent) or other small changes (about 15-30 percent), but invariably they prevent synthesis of functional dystrophin protein.

22. Dystrophin plays a key structural role in muscle fiber function. In healthy muscle, dystrophin interacts with other proteins at the cell membrane to stabilize and protect the cell during regular activity involving muscle contraction and relaxation.

23. Patients with DMD produce little or no dystrophin in their muscle. Without dystrophin, normal activity causes excessive damage to muscle cells, and they are ultimately replaced by fibrotic tissue and fat, leading to a progressive loss of function.

24. Despite a great medical need for a cure, as of this date there are limited FDA approved therapies for treating DMD. The FDA has granted accelerated approval of four antisense oligonucleotides for exon skipping: (1) eteplirsen (Exondys 51) for skipping for Exon 51 of the

dystrophin pre-mRNA; (2) golodirsen (Vyondys 53) for skipping Exon 53 of the dystrophin pre-mRNA; (3) viltolarsen (Viltepso[®]) for skipping Exon 53 of the dystrophin pre-mRNA; and (4) casimersen (Amondys 45) for skipping Exon 45 of the dystrophin pre-mRNA. The FDA has also granted accelerated approval of Delandistrogene moxeparvovec (Elvidys) a gene therapy treatment for pediatric patients 4 through 5 years of age.

B. Becker Muscular Dystrophy

25. Becker Muscular Dystrophy (“BMD”) also results from mutations in the dystrophin gene, but symptoms in patients with BMD are milder and typically display a later, and much slower, rate of progression. BMD symptoms are variable but include slowly progressing muscle weakness, with the ability to walk typically continuing well into adulthood.

26. Patients with BMD generally carry a mutation (or several) that causes the body to make a functional, though shorter, form of the dystrophin protein. Errington et al., J. Gene Med. 2003; 5:518-27 (“Errington 2003”) at 524. Many BMD patients make dystrophin protein missing portions, even substantial portions, of the central domain of the dystrophin protein. This means that loss of even a substantial part of the central domain of the dystrophin protein can occur with relatively little impact on protein function. Douglas & Wood, Molecular & Cellular Neurosci. 2013; 56:169-85 (“Douglas & Wood 2013”) at 172.

27. This contrasts with mutations causing DMD, which typically disrupt the mRNA reading-frame or prevent synthesis of either end of the dystrophin protein. Errington 2003 at 524.

28. The goal of exon skipping therapies to treat DMD, the technology at issue in these proceedings, is to correct for specific genetic mutations that cause DMD and restore the patient’s ability to make a partially functional, though shorter, form of the dystrophin protein, similar to

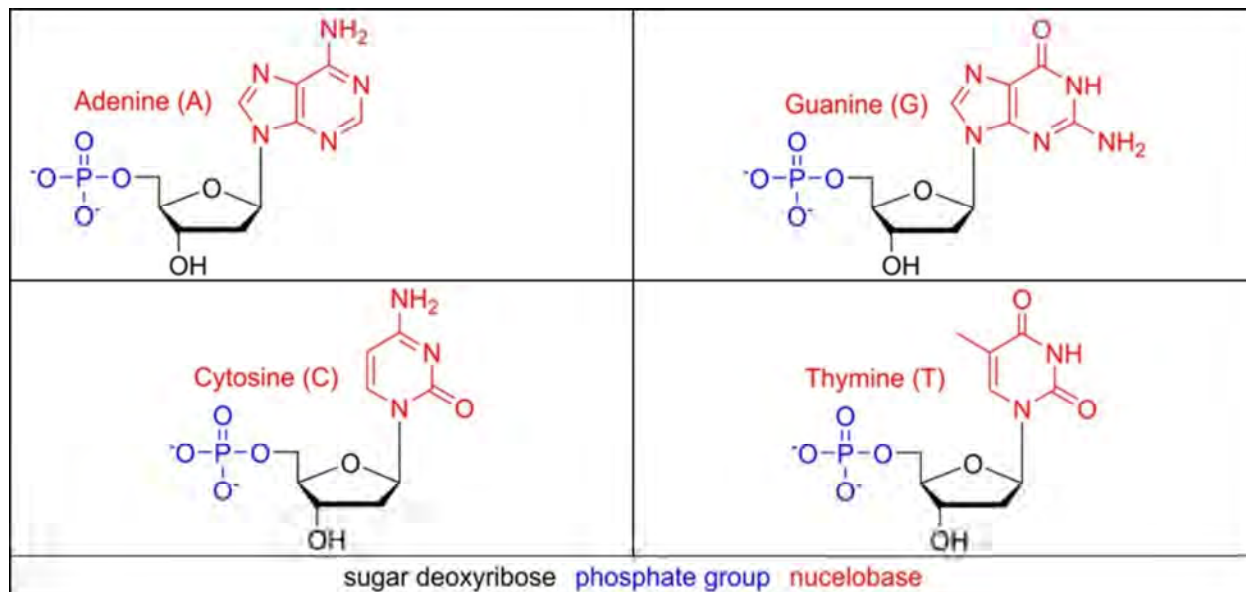
dystrophin protein found in BMD patients. To understand how this works, some additional background is required.

C. Transcription, Splicing, and Translation

29. Mammalian cells are surrounded by a membrane and usually contain an inner body, the nucleus, which is also surrounded by a membrane. Deoxyribonucleic acid (“DNA”), found in the nucleus of cells, carries the genetic information used in the development and functioning of all living organisms.

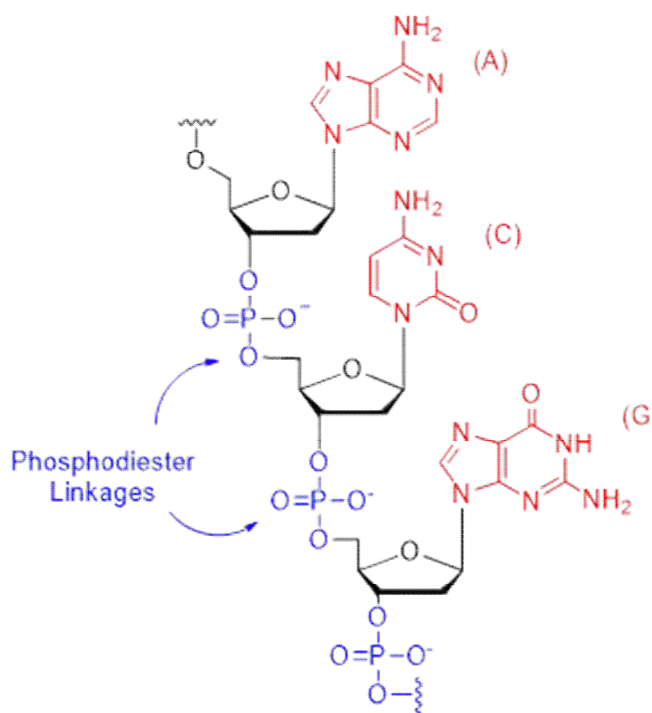
30. DNA is constructed from four main nucleotide building blocks. Each of these nucleotides contains a phosphate group linked to a five-carbon-atom deoxyribose sugar group, which in turn is joined to one of four possible nucleobases: adenine (A), cytosine (C), guanine (G), and thymine (T).

31. The figure below shows each of the four naturally occurring nucleotides of DNA, all of which contain the sugar deoxyribose, a phosphate group, and a nucleobase.



32. The nucleobases “adenine” and “guanine” are examples of purines, which are bicyclic aromatic compounds. The nucleobases “cytosine” and “thymine” are examples of pyrimidines, which are monocyclic aromatic compounds.

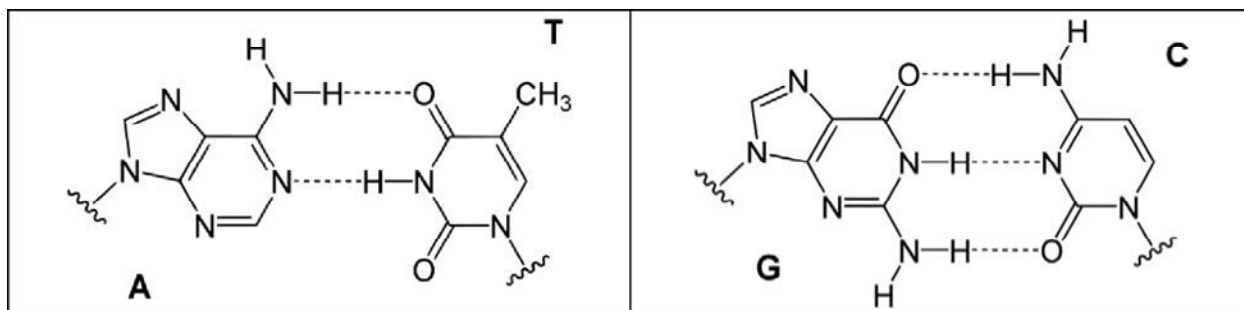
33. Nucleotides in DNA are joined together by internucleotide linkages between the sugar of one nucleotide and the phosphate of the next. These bonds are called phosphodiester linkages. The following figure shows a portion of a DNA polynucleotide chain, including the phosphodiester linkages that connect the nucleotides.



As can be seen above, each nucleotide in the chain contains a nucleobase and a sugar, sometimes called the chemical backbone, and each nucleotide is connected by the internucleotide linkage.

34. The four naturally occurring DNA nucleobases form “complementary” pairs that interact through hydrogen bonds, with the purines interacting with the pyrimidines. Adenine (A) interacts with thymine (T), and cytosine (C) interacts with guanine (G). This is called Watson-

Crick base pairing and is illustrated in the following figure for A-T and G-C pairing, with dotted lines representing hydrogen bonds:



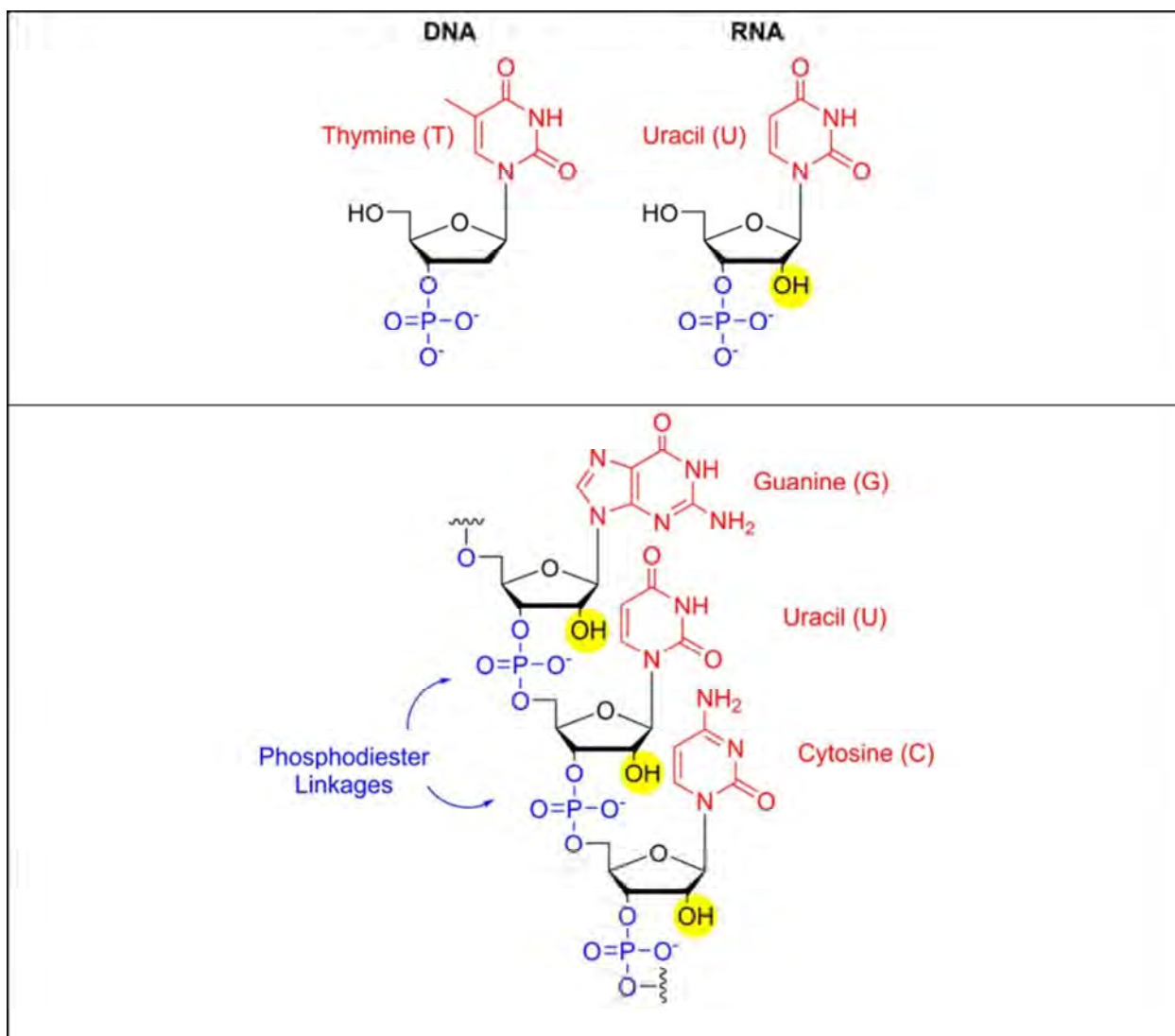
35. Base pairing allows two complementary DNA strands to form a double helix. The complementary nature of base pairing also allows cells to make copies of DNA strands because the sequence of nucleobases on one strand dictates the nucleobase sequence of the other strand.

36. In cells, DNA is organized into very long structures called chromosomes. Human chromosomes contain about 22,000 genes, each of which encodes a different protein. Despite this, substantial portions of our chromosomes do not encode proteins. It is estimated that about 1.5% of the human genome consists of protein-coding regions, with the remainder consisting of non-coding sequences.

37. To create proteins, DNA in the nucleus needs to be “transcribed” into messenger ribonucleic acid (“mRNA”).

38. Like DNA, RNA is constructed from four nucleotide building blocks. Each RNA nucleotide contains a phosphate group linked to a ribose sugar group, which in turn is linked to one of four possible nucleobases: adenine (A), cytosine (C), guanine (G), or uracil (U). Naturally occurring RNA therefore chemically differs from DNA in that (1) the sugar group is ribose instead of deoxyribose and (2) RNA contains the pyrimidine nucleobase uracil (U) instead of the nucleobase thymine (T). The following figure shows a portion of an RNA polynucleotide chain, including the phosphodiester linkages that connect each ribonucleotide. As can be seen below,

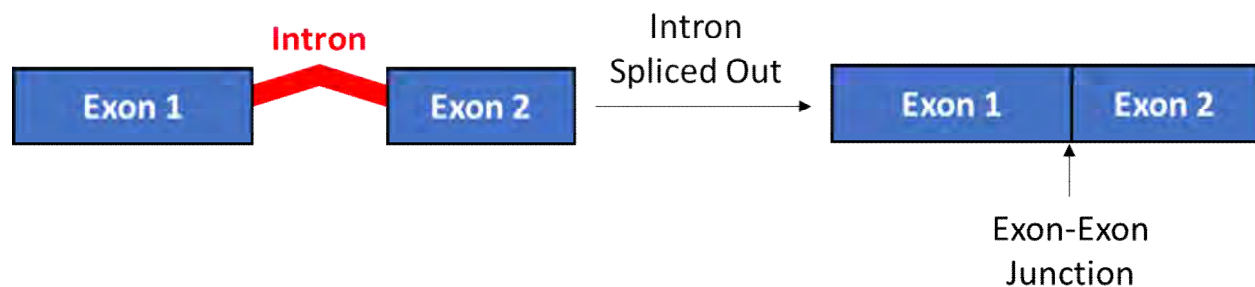
each nucleotide in the RNA chain contains a nucleobase and a ribose sugar, and each nucleotide is connected by an internucleotide linkage.



39. Transcription of DNA into mRNA is a multi-step process. In the nucleus, genes are transcribed to form a precursor mRNA (or “pre-mRNA”), with the nucleobase sequence of the DNA dictating the nucleobase sequence of the pre-mRNA. These nuclear pre-mRNAs include “exons,” segments that encode protein that are typically retained in the mature mRNA, as well as “introns,” which are typically removed from the pre-mRNA in a process called “splicing.” Splicing

of the pre-mRNA transcript occurs in the nucleus shortly after transcription, and is mediated by a large complex of proteins and RNAs called the “spliceosome.”

40. A schematic of two exons separated by a single intron is shown in the following figure. During the splicing process, the intron (or “intervening sequence”) is removed (spliced) from the mature mRNA.



41. Introns contain a donor site at one end and an acceptor site at the other. Both the donor and acceptor sites are required for splicing. Certain other nucleotide sequences, called intronic or exonic splicing enhancers or intronic/exonic splicing silencers, also can influence splicing efficiency.

42. Exon skipping, the subject of the technology at issue in this litigation, involves the therapeutic manipulation of splicing events. As explained in the following section, exon skipping aims to manipulate pre-mRNA splicing using antisense oligonucleotides (“AONs”).

43. Following pre-mRNA splicing, the “mature” mRNA is transported out of the nucleus. That mRNA can then be “translated” into a protein.

44. It is useful to consider the example of dystrophin in considering these concepts. The dystrophin gene is one of the longest human genes, covering about 2,400,000 nucleotides of the X chromosome. In a healthy individual, the transcription machinery in the nucleus produces

full-length dystrophin pre-mRNA transcripts measuring about 2,400,000 nucleotides. These full-length pre-mRNA transcripts contain 79 exons and 78 introns.

45. The splicing machinery in the nucleus then removes all 78 introns, many of which are very large, creating a mature transcript of about 14,000 nucleotides. It is the splicing machinery that therefore generates the actual coding region for the protein by splicing together the exons from the pre-mRNA.

46. Healthy patients produce at least seven shorter forms of dystrophin protein, each transcribed using an alternative promoter. There are also likely to be considerably more forms of dystrophin owing to the presence of multiple alternative splicing events. Nevertheless, the full-length skeletal muscle isoform encodes a protein that is 3,685 amino acids in length. Douglas & Wood 2013 at 170.

47. As mentioned, after splicing the dystrophin mRNA is transported out of the nucleus into the cytoplasm, where the dystrophin protein is produced.

D. Conceptual Framework for Treating DMD by Exon Skipping

48. As discussed above, loss of a portion of the dystrophin protein, even a substantial portion of the dystrophin protein, can occur with relatively little impact on protein function.

49. However, in many patients with DMD, deletion of an exon or exons produce a “frame shift” such that the remaining exons are misaligned or “out of frame,” interrupting proper translation of the genetic code into protein. Patients with these mutations typically produce little or no functional dystrophin protein.

50. Exon skipping seeks to realign the remaining exons, making them “in-frame,” by causing the cellular machinery to “skip” over an exon in the pre-mRNA. By restoring the reading

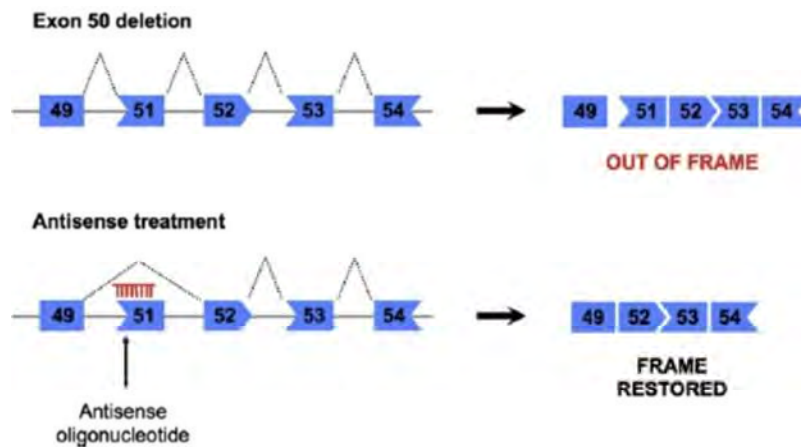
frame, the cellular machinery is able to translate the mRNA into a functional, though shorter, form of dystrophin.

51. Exon skipping therapies therefore must be patient specific, in that a patient's specific mutation will determine which exon-skipping therapy will provide a therapeutic benefit. Thus, although a cluster or collection of mutations can all be treated by skipping the same exon, each patient must be evaluated to determine if he or she can be treated by skipping a particular exon. With respect to patients with DMD, while deletions are spread across the 79 DMD exons, there are specific "hotspot" regions where deletions are particularly common, such as between exons 45 and 55 where around 70% of deletions are located. Douglas & Wood 2013 at 172. Patients carrying these mutations are promising candidates for exon therapy treatments.

52. Other patients with DMD carry "nonsense" mutations that cause premature termination of the dystrophin protein. Nonsense mutations are point mutations that introduce a premature "stop" codon, preventing translation of full-length protein. These patients also typically produce little or no functional dystrophin protein. Nonsense mutations are believed to account for around 15% of known DMD mutations. Exon skipping therapies could also be designed to skip exons containing these nonsense mutations, such that cells would make functional, though shorter, dystrophin protein. Of course, in these cases the reading frame must still be maintained, so single exon skipping for these mutations is limited to those exons that are not frame-shifted. However, this would still apply to around 47% of patients with dystrophin nonsense mutations. Douglas & Wood 2013 at 172.

53. The following figure shows the principle of exon skipping in DMD. In this example, exon 50 of DMD is deleted, causing a frameshift in the resulting spliced mRNA. Addition of an AON (shown in red and annotated with an arrow) that specifically binds to a target sequence

within exon 51 mediates skipping of this exon by the spliceosome (the cellular machinery responsible for exon skipping), such that exon 49 is spliced directly to exon 52. Although the resulting mRNA is missing the portion of the dystrophin protein encoded by exons 50 and 51, this has a minimal effect on overall protein function.



Douglas & Wood 2013 at 173.

54. Available data suggests that up to approximately 80% of DMD patients carry mutations potentially amenable to exon skipping. Studies show that approximately 13% of DMD patients are candidates for treatment by exon 51 skipping, and another 8% of patients are candidates for treatment by exon 53 skipping. Douglas & Wood 2013 at 172.

E. Antisense Oligonucleotides for Exon Skipping

1. Requirements for Exon Skipping

55. AONs are the primary therapeutics being investigated for exon skipping. AONs are single-stranded, short lengths of nucleotides. AONs have molecular weights 10-20 times that of a small molecule and, as explained below, are subject to a multitude of possible chemical modifications.

56. To be effective therapeutic agents for modulating splicing, AONs ideally possess a number of intrinsic properties.

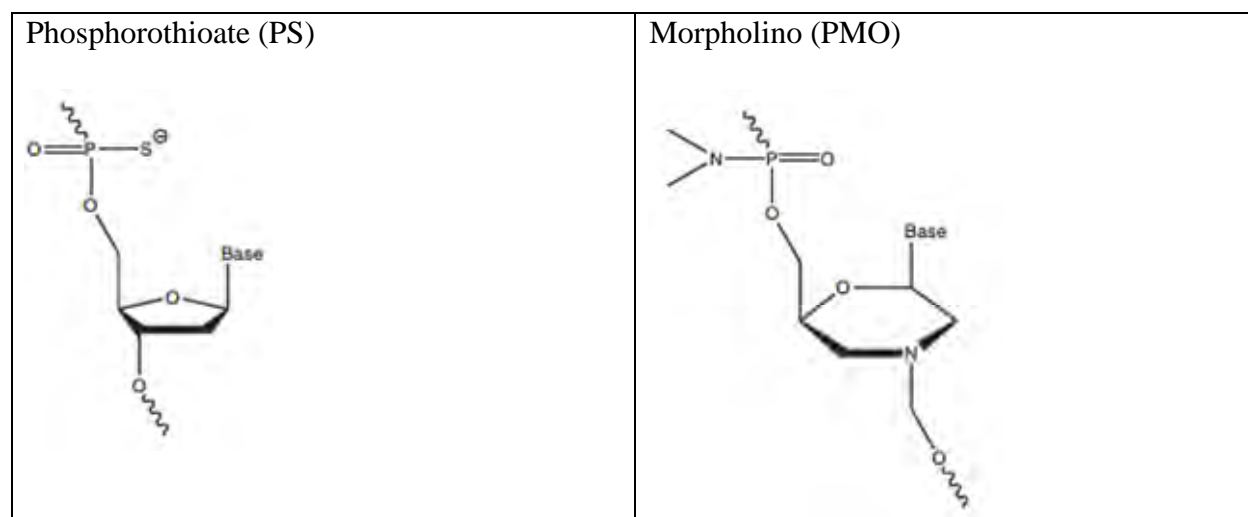
- First, the AON should bind in a sequence-specific manner to the target pre-mRNA: the higher the specificity, the less chance of unwanted off-target effects.
- Second, the AON needs to bind to its target with appropriate affinity. If the AON binds poorly, it will not induce exon skipping (or will not induce sufficient exon skipping). On the other hand, if the AON binds too tightly, it will not detach quickly enough (or at all) from a particular target transcript. Detachment is essential because dystrophin pre-mRNAs are constantly being generated in muscle cells. If detachment is too slow (or does not occur), the AON will not induce sufficient exon skipping.
- Third, the AON needs to be delivered effectively to the appropriate tissue type or cells upon administration.
- Fourth, because the body contains numerous proteins that degrade oligonucleotides (called “nucleases”), an exon skipping AON should be resistant to nuclease degradation to allow it to reach its desired target intact and to maximize its potential duration of action once there.
- Fifth, upon reaching the appropriate tissue type or cells, the AON needs to penetrate into the cell and also into the appropriate intracellular compartment. Because pre-mRNA splicing takes place in the nucleus, it is critical that an exon skipping AON localize to the nucleus once it is taken up.
- Sixth, once complexed with the target pre-mRNA, the AON/RNA duplex should be resistant to the nuclease RNase H, which normally degrades RNA bound up in duplexes.
- Seventh, the AON should not be toxic.
- Eighth, the AON should have favorable pharmacokinetic and pharmacodynamic properties.

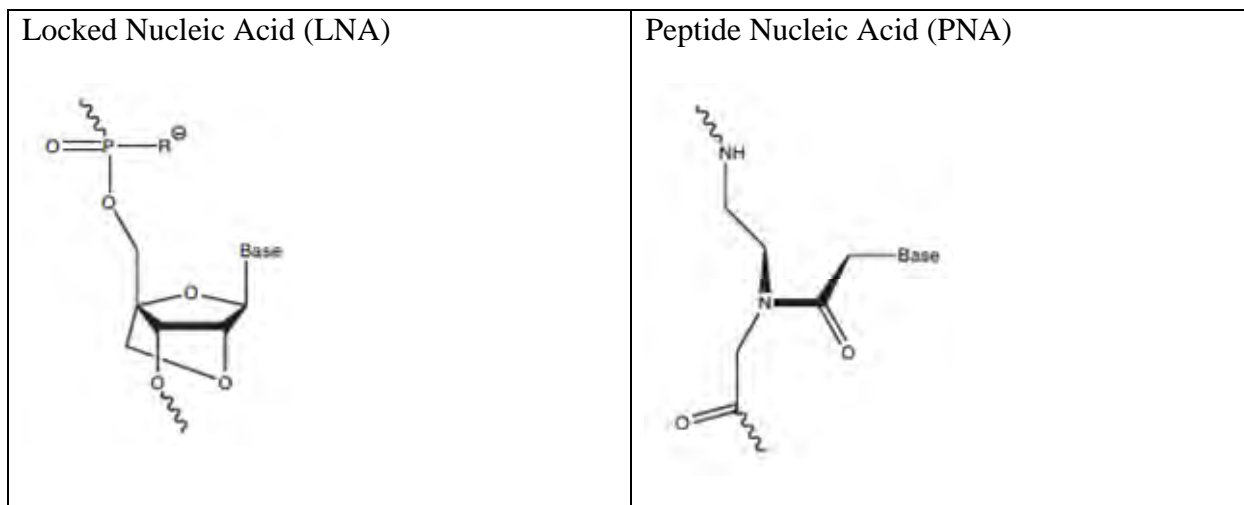
Douglas & Wood 2013 at 173.

2. Prominent Classes of AON Proposed for Exon Skipping

57. Because chemical changes can be made at almost every position of the nucleobase, chemical backbone, and internucleotide linkages, the number of possible modifications to naturally occurring DNA/RNA nucleotides is immense. In an effort to cope with the many requirements for exon skipping, scientists have explored many different AON chemistries, including AONs with modifications to the nucleobase, the backbone, the internucleotide linkages, and combinations of each.

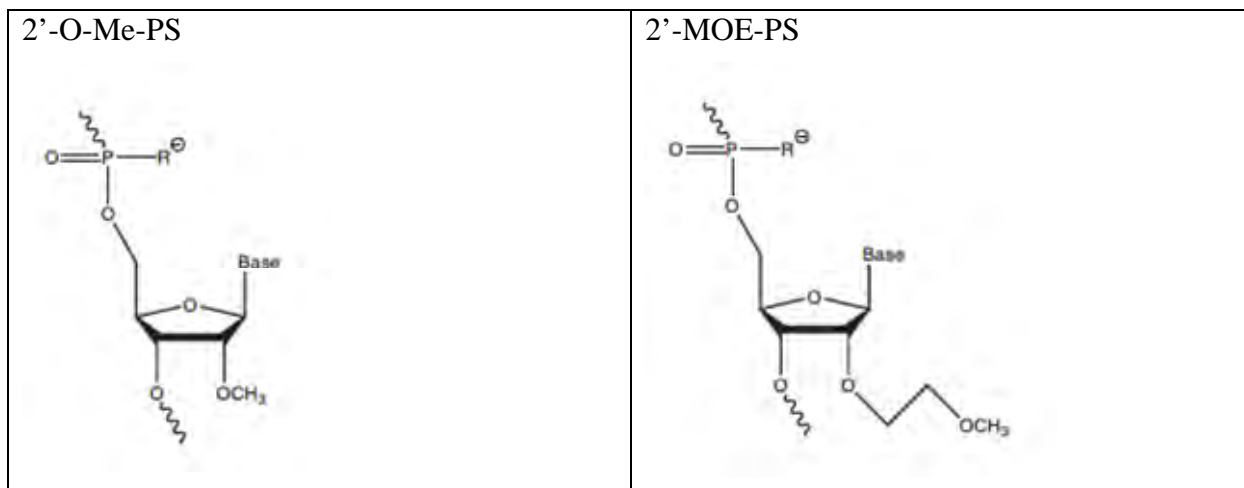
58. AON chemistries can vary significantly from naturally occurring nucleotides. However, they preserve the ability to form Watson-Crick base pairs with pre-mRNA through the maintenance of nucleobases (sometimes modified) in the correct sequence orientation. Although not exhaustive, a selection of significant types of AON chemistries proposed for exon skipping is illustrated in the following figure and discussed below. These include phosphorothioate (“PS”), morpholino (“PMO”), 2’-O-methylphosphorothioate (2’OMe), 2’-O-methoxyethyl (MOE), locked nucleic acid (“LNA”), and peptide nucleic acid (“PNA”).





Jarver et al., *Nucleic Acid Ther.* 2014; 24(1):37-47 (“Jarver 2014”) at 38.

59. Phosphorothioates are chemically similar to RNA, but the non-bridging oxygen atom of the phosphate group of RNA is replaced by a sulfur atom. As illustrated in the following figure, commonly used phosphorothioates include 2'-O-methyl phosphorothioate (“2'-O-Me-PS”), which have a methyl group attached to the oxygen atom at the 2' position of the ribose ring, as well as 2'-O-methoxyethyl phosphorothioate (“2'-MOE-PS”), which add a methoxy group instead of a methyl group. These modifications confer some nuclease resistance.



Jarver 2014 at 38.

60. Phosphorothioates retain a negative charge. As explained below, this property aids in their solubility, but it also makes them “sticky,” meaning that they have a tendency to nonspecifically bind proteins, which can prove problematic for efficient delivery *in vivo* and lead to toxicity.

61. Phosphorodiamidate morpholino oligomers (called “morpholinos” or “PMOs”) have six-membered morpholine rings in place of ribose. Additionally, each “nucleotide” is joined together by phosphorodiamidate linkages rather than phosphodiester linkages.

62. Morpholinos are nuclease and RNase H resistant and have no charge on their backbone at physiologic pH. Morpholinos have minimal toxicity and are very stable, as the majority of administered compound is excreted essentially unchanged in urine. Unlike phosphorothioates, they have no net electrical charge and therefore do not tend to interact with non-target molecules. Douglas & Wood 2013 at 174.

63. Peptide nucleic acids (“PNAs”) replace the sugar group of DNA and RNA with repeating N-(2-aminoethyl)-glycine units linked by peptide bonds (which are typically found in proteins, not nucleic acids).

64. Like morpholinos, PNAs are uncharged at physiologic pH. PNAs are also resistant to enzyme degradation. PNAs tend to have very high target binding affinity, but PNA/RNA mismatches are more destabilizing than a similar mismatch in an RNA/RNA duplex. Unmodified PNAs cannot readily cross cell membranes, and PNAs are less soluble than RNA oligonucleotides. Braasch & Corey, *Biochem.* 2002; 41(14):4503-10 (“Braasch & Corey 2002”) at 4507-4508.

65. As compared to RNA, locked nucleic acids (“LNAs”) contain a bond connecting the 2'-oxygen of the ribose with the 4'-carbon. This bond “locks” the sugar portion of the

nucleotide in a particular confirmation, and as a consequence LNAs are conformationally inflexible. Braasch & Corey, *Chem & Biol.* 2001; 8:1-7 (“Braasch & Corey 2001”) at 1.

66. LNAs have an extremely high affinity for RNA and DNA. Additionally, LNAs have shown low toxicity in clinical trials and are RNase H resistant. Braasch & Corey 2001 at 3.

67. Many modifications have been and can be made within these commonly used frameworks. For example, non-natural modified nucleobases could be used in place of A, C, G, T, and U. Such well-known nucleobases include, by way of example only, 5-substituted pyrimidines; 6-aza-pyrimidines; and N-2, N-6, and O-6 substituted purines.

68. Such non-natural nucleobases may bind more or less tightly than their natural equivalent, and therefore may alter the affinity of a particular AON for its target. For example, 5-methyl-cytosine increases duplex stability as compared to unmodified cytosine (C). Koshkin et al., *Tetrahedron* 1998; 54:3607-30 at 3613.

69. Researchers have also investigated AONs made with internucleotide linkages other than those called out above. These include, by way of example only, phosphotriester, methylphosphonate, phosphoroamidate, carbonyl, and sulfonyl linkages. Summerton & Weller, *Antisense & Nucleic Acid Drug Dev.* 1997; 7:187-95 at 189. Changing these internucleotide linkages can alter binding specificity and affinity, nuclease resistance, delivery to target tissue, uptake into cells, and intracellular targeting.

70. AONs can also be made that are synthesized partly with one chemistry and partly from another. These are called “chimeric” or “hybrid” AONs. For example, “gapmers” have been studied that contain a central region of 2'-O-Me-PS nucleotides flanked by LNAs. Braasch & Corey 2002 at 4509. Similarly, LNA/2'-O-Me RNA chimeras have been developed to improve the potency and specificity of their action. Braasch & Corey 2002 at 4509.

71. The stereochemistry of AONs can also be modified to change the pharmacologic properties. Iwamoto et al., Nature Biotechnol. 2017; 35:845-851 at Abstract. PS linkages can have two different stereochemical conformations. Accordingly, a 20mer length PS AON has the possibility of 2^{19} different stereochemical conformations.

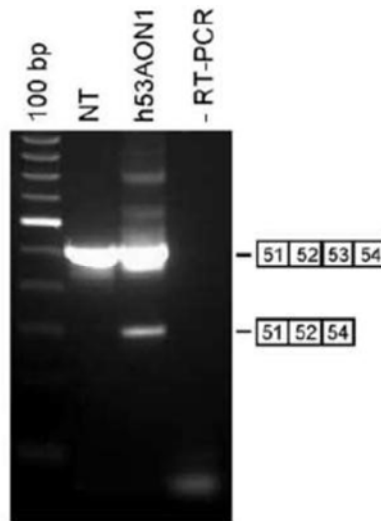
3. *In vitro* skipping assays

72. Exon skipping is typically studied in cultured cells. Some studies use primary human myoblasts (muscle cells) isolated from human muscle biopsies of affected or non-affected individuals. Other studies use human muscle tumor cell lines.

73. Regardless of the cell source, the cultured cells are often “transfected” with relatively high concentrations of AONs, often ranging from 100 nM to 1 mM. To facilitate cell transfection, chemical reagents such as polyethylenimine (“PEI”) or lipofectamine are added to the cell cultures to help the AON penetrate the cellular membrane. These agents are relatively toxic and consequently are not suitable for use in patients. Alternatively, cell cultures can be transfected by nucleofection, which uses electricity to disrupt the integrity of the nuclear membrane to enable the AONs to directly enter the nucleus of target cells. Although these transfection techniques can be used *in vitro*, they cannot for safety reasons be used for delivering the AON drug to patients.

74. Although different methods can be used to monitor exon skipping *in vitro*, the primary method is called RT-PCR. In this technique, RNA is isolated from cultured cells following transfection with the AON. An oligonucleotide adjacent to the target exon is used to “prime” the enzyme reverse transcriptase (“RT”), which converts the RNA into a complementary DNA (cDNA). That cDNA is then used as a template for exponential amplification using polymerase chain reaction (“PCR”). If the AON induces exon skipping, the PCR product will be shorter than

it otherwise would. This is depicted in the following figure, which shows that the AON induces formation of a smaller band having faster mobility than the control sample.



Adapted from Aartsma-Rus et al., *Neuromuscular Disorders* 2002, 12:S71-S77 (“Aartsma-Rus 2002”), Fig. 1(i). In this example, exon skipping appears relatively inefficient, as the presence of the large upper band reveals that most of the product contains exon 53. Notably, RT-PCR provides no information as to whether or not dystrophin protein is produced. In the case of DMD, it is the protein, not the RNA, that is necessary to restore muscle function.

F. In Vitro Exon Skipping Experiments Are Unpredictable

75. Even in these relatively controlled *in vitro* skipping studies, exon skipping is unpredictable.

76. In a 2001 publication, van Deutekom and coworkers found that, “[t]he efficacy of AONs is largely determined by their binding affinity for the target sequence. Due to base composition and pre-mRNA secondary or tertiary structure, *it is difficult to predict which AONs are capable of binding the target sequence.*” van Deutekom et al., *Hum. Mol. Genet.* 2001; 10(15):1547-54 (“van Deutekom 2001”) at 1548 (emphasis added).

77. Similarly, in 2002, Aartsma-Rus and colleagues concluded as follows: “We therefore ***have no insight*** into the actual position of the targeted sequence within the completely folded RNA structure. Its accessibility, and thus ***the effectivity of any designed AON, will therefore still have to be tested empirically in the cells, as was done in this study.***” Aartsma-Rus 2002 at S76 (emphasis added).

78. A 2007 paper by van Deutekom and colleagues states that “several years after the first attempts at dystrophin exon skipping with [antisense oligonucleotides], ***there are still no clear rules to guide investigators in their design***, and in mouse and human muscle cells *in vitro* ***there is great variability for different targets and exons.***” Arechavala-Gomez et al., Human Gene Ther. 2007; 18:798-810 (“Arechavala-Gomez 2007”) at 807 (emphasis added).

79. In 2009, the Aartsma-Rus and colleagues wrote that while existing software programs can facilitate exon skipping AON design, “in general ***a trial and error procedure*** is still involved to identify potent AONs.” Aartsma-Rus et al., Mol. Ther. 2009; 7(3):P548-53 (“Aartsma-Rus 2009”) at 548 (emphasis added).

80. There are numerous examples of this *in vitro* unpredictability. For example, van Deutekom and coworkers reported in 2001 that mAON9 induced exon skipping in cultured mouse muscle cells, but mAON8 did not. These AONs were both made with the same 2'-O-Me-PS chemistry and both apparently bound to their target sequences. Moreover, these AONs substantially overlapped, as both contained the nucleobase sequence “UUAGCUGCUGC” as well as additional nucleobases complementary to the mouse dystrophin gene. Yet one induced skipping of exon 46, and the other did not. van Deutekom 2001 at 1548.

81. Another example was published by Wu and coworkers in 2011. These researchers screened a series of AONs covering more than two thirds of human dystrophin exon

50 and two flanking intron sequences. A subset of the tested sequences, all made with 2'-O-Me-PS chemistry, is shown in the Table below.

Name	Target ¹	2'-O-Me-PS AON Sequence	Length	Effect
AO3PS	-19+1	UCUUUAAACAGAAAAGCAUAC	20	-
AO4PS	-19+3	CCUCUUUAAACAGAAAAGCAUAC	22	4%
AO5PS	-19+8	AACUCCUCUUUAAACAGAAAAGCAUAC	27	21%
AO6PS	-19+13	CUUCUAAACUCCUCUUUAAACAGAAAAGCAUAC	32	3%

Wu et al., PLoS One 2011; 6(5):e19906 (“Wu 2011”) at 4. The 20-mer AO3PS induced no detectable exon skipping. The 22-mer AO4PS, differing only in having two additional nucleotides complementary to the *DMD* gene, induced detectable exon skipping in 4% of cells. Adding an additional five nucleotides increased exon skipping to 21%. However, adding five more nucleotides largely abrogated this effect. Consistent with this Aartsma-Rus and coworkers similarly noted in a 2009 publication that increasing AON length could decrease exon skipping efficiency. Aartsma-Rus 2009 at 552.

82. Similarly, Meloni and colleagues found in a 2007 publication that “AOs of 25-31 nucleotides are generally more effective at inducing exon skipping than shorter compounds. However, there appears to be an upper limit in optimal length, which may have to be established on a case-by-case basis.” Harding et al., Mol Ther. 2007; 15(1):157-166 at 157. The observations on AON length versus skipping efficiency reported by Wu et al. are consistent with my experience. AONs have an optimal length, which is a result of a number of factors including nucleotide sequence, chemical modifications, and target accessibility, and when that length is either not reached or is exceeded the skipping efficiency drops off.

¹ The Target column shows the coordinates of the target site relative to the pre-mRNA sequence. “+” represents an exonic position and “-” represents an intronic position, with the numbers representing the first and last nucleotides AON target sequence. Wu 2011 at 4.

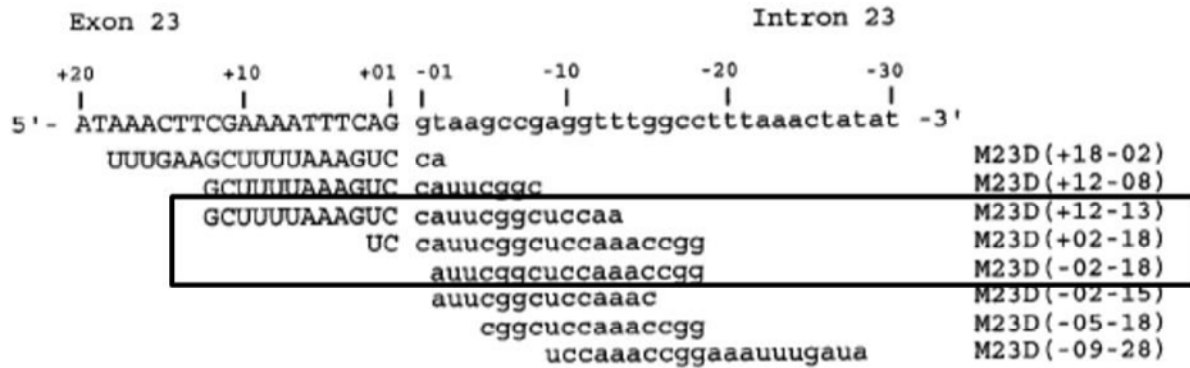
83. Consequently, I would expect that the great majority of longer AONs, on the order of 50 nucleotides or longer, will not efficiently induce skipping for a number of reasons, if they induce it at all, to be useful as therapeutic agents for treating DMD. Those reasons include access to the complementary sequence in the pre-mRNA, binding affinity of the AON for the complementary sequence, and the ability to transfect longer AONs into cells and delivery longer AONS effectively to muscle tissues in DMD patients.

84. Heemskerk and coworkers also highlight this unpredictability. Heemskerk et al., J. Gene Med. 2009; 11:257-66 (“Heemskerk 2009”) at 259-60. For example, Heemskerk analyzed exon skipping by RT-PCR of mouse exon 23 with a “short” 2’-O-Me-PS AON that was 20 nucleotides in length, a “long” 2’-O-Me-PS AON that was 25 nucleotides in length, and the “long” AON made as a PMO. The sequences of these AONs are shown below:

Name	2’-O-Me-PS AON Sequence	Length
m23 AON5’ss	UCCAUCGGCUCCAAACCGG	20
m23 AON5’ss long	UAAAGUCCAUCGGCUCCAAACCGG	25
m23 PMO5’ss	TAAAGTCCATTCGGCTCCAAACCGG	25

According to the authors, “[t]he PMO induces significantly higher levels of exon skipping than both [2’-O-Me-PS] AONs.” *Id.* at 259. But “[t]he long [2’-O-Me-PS] is significantly less efficient than the short version.” *Id.* at 259. This data shows the complex interactions between nucleotide length, nucleotide sequence, internucleotide linkages, and chemical backbone, and reinforces the need for empirically testing each chemically distinct AON.

85. Another study, authored by Wilton’s group at UWA, examined skipping of exon 23 from the mouse DMD gene by RT-PCR following transfection with a series of overlapping 2’-Me-O-PS AONs, as shown in the following figure. Of the AONs tested, only M23D(+12-13), M23D(+02-18), and M23D(-02-18) were effective in inducing detectable exon skipping. Mann et al., J Gene Med 2002; 4:644-54 at 647.



Id. at 646. Notably, the shorter AON M23D(-02-18), which is only 17 nucleotides in length, was particularly efficient at inducing skipping and was reported to induce exon skipping at concentrations as low as 5 nM. The authors concluded that they could improve “the efficiency of the technique” by “reduc[ing] the size and the effective dose of the AO[N]s” examined. *Id.* at 644.

86. Arechavala-Gomez and colleagues investigated eight specific AON sequences targeting human DMD exon 51 using two different chemical forms (2'-O-Me-PS and morpholino) in human muscle cells, human muscle explants, and human muscle explants from patients with DMD. Arechavala-Gomez 2007 at 798. Five AONs targeting the 5' splice site were "surprisingly" determined to be largely ineffective at inducing skipping of exon 51. *Id.* at 803. Three other overlapping sequences complementary to portions of exon 51 were each capable of inducing exon 51 skipping in cells from healthy patients, including sequence B30 (targeting +66+95) and A20 (targeting +68+87). *Id.* at 803. But B30 induced significantly better exon skipping in cells derived from a DMD patient with a deletion in exons 48 and 50 than A20. *Id.* at 805 ("the skip achieved with AO A20 was less efficient"). Similarly, B30 induced significantly better skipping in cells derived from a DMD patient with a deletion in exon 50 than A20. *Id.* at 805 ("the percentage of the skip was 13% for AO[N] A20 and 73% for AO[N] B30.") This shows that both nucleobase sequence and target cell type influence exon skipping.

87. Several factors complicate analysis of these and other studies. First, different AONs show varying effectiveness in different cell types (for example, cells from healthy individuals or DMD patients), as was observed in the Arechavala-Gomeza publication discussed above. Those authors report that “the very nature of this targeted exon skipping approach makes it impossible to test many of the specific sequences to be used in humans in healthy volunteers, because of the potential for disrupting the intact dystrophin open reading frame, leading to nonfunctional dystrophins.” Arechavala-Gomeza 2007 at 808; *see also* Aartsma-Rus et al., Hum. Mol. Genet. 2003 12(8):907-14 (“Aartsma-Rus 2003”) at 909. Similarly, an AON that appears to have activity in one type of patient cells, such as skeletal muscle, may not have activity in other types of patient cells, such as cardiac muscle for reasons such as the ability of AONs to penetrate the respective cell types. Douglas & Wood 2013 at 179.

88. Second, *in vitro* exon skipping studies often use transfection reagents such as PEI that in essence form nanoparticles. It is impossible to know the actual AON dose administered to cells because these particles form suspensions. Complicating matters further, different reagents are used for transfecting cells with AONs having different chemical backbones, undermining “dose” and “efficiency” comparisons across studies. For example, we have recently shown that the *in vivo* activity of anionic AONs is correctly modeled *in vitro* only when using gymnotic delivery, that is, transfection of the AON into cells in the absence of a delivery agent like PEI. Hammond et al., Mol. Ther. Nucleic Acids. 2014; 3:E212 at 1.

89. Third, even *in vitro*, it is difficult to extrapolate results from one AON class (for example, a morpholino) to another. Because different AON chemistries influence binding affinity, each type of AON will have different binding characteristics, even with identical nucleobase sequences. This is reflected in the optimal lengths observed for AONs of different types: although

there may be exceptions, in my experience, the typical length for an exon skipping 2'-O-Me-PS is 18-20 nucleotides; the typical length for a morpholino is 22-30 nucleotides; the typical length for a PNA is 22-25 nucleotides; and the typical length for an LNA is 13-18 nucleotides. Thus, if one attempted exon skipping with a morpholino AON using a nucleobase sequence that induced *in vitro* skipping when contained in a LNA AON, it would be unlikely to induce *in vitro* skipping as effectively. Conversely, if one attempted exon skipping with a LNA AON using a nucleobase sequence that induced *in vitro* skipping with a morpholino AON backbone, it also would be unlikely to induce *in vitro* skipping as effectively, and moreover would likely bind to other parts of the genome because of the relatively high binding affinity of LNAs.

G. Mismatches Unpredictably Alter Efficacy of AON Exon Skipping Activity

90. A mismatch occurs when there is a discrepancy in the Watson-Crick base pairing of the AON and the target pre-mRNA. Several studies have evaluated the effects of mismatches on exon skipping.

91. One study evaluated AONs designed against exon 19 of the mouse DMD gene in inducing skipping of the human DMD gene. With the exception of one AON (out of nine) containing seven mismatched bases, all mouse-specific AONs induced exon 19 skipping in human cells. However, 20- to 100-fold higher concentrations of mismatched 2'-O-Me-PS AONs were required as compared to perfectly complementary AONs. This was believed to result from the reduced annealing potential caused by the mismatched nucleobases. Errington 2003 at 525.

92. Another study evaluated a panel of AONs designed to skip exon 25 from human dystrophin in normal and dystrophic patient cells. While the investigators expected that a single mismatch mutation would compromise AON binding and efficiency, the mismatched AON actually induced exon skipping *more* efficiently than a perfectly complementary AON. Fragall et

al., BNC Medical Genetics 2011; 12:141 at 5. As stated by the investigators, “most unexpectedly, H25A(+95+119), which annealed across the insertion and was therefore mismatched, induced the most robust exon skipping.” *Id.* In some instances, mismatches can therefore increase exon skipping efficiency.

93. Another study revealed unpredictable interactions between mismatches and AON backbone type. There, the investigators investigated skipping of mouse *DMD* exons by AONs designed against the human *DMD* gene. The authors explained:

For the longer [2'-O-Me-PS] and PMO [morpholino] AONs, there were at least two mismatches with the mouse sequence. For the [2'-O-Me-PS], these mismatches almost completely abolished AON efficacy. By contrast, two of the four PMOs tested induced skipping of the murine exon. For exon 51, this aspecific skipping was at a lower level than that observed for human exon 51. However, for exon 45, the skipping levels of the mouse and human exons were similar.

Heemskerk 2009 at 264. Thus, some mismatches abolished AON activity, while others did not. And the effect of the mismatches in some cases depended upon the particular chemical backbone used to construct the AON. Notably, the authors warn that the chance of mistargeting “will increase significantly” if AONs are unable to discriminate between their target sequence and a sequence containing two mismatches. *Id.* at 264.

94. Mismatches between an AON and its target sequence in the dystrophin pre-mRNA, therefore, will affect the ability of the AON to induce exon skipping, but one cannot predict *a priori* the impact any given mismatch(es) will have on exon skipping activity, whether positive or negative. This was true in June 2004 and is still true today.

H. Additional Challenges with *In Vivo* Exon Skipping

1. AONs had yet to fulfill their potential as of 2005

95. As of 2005, no exon skipping drug had been granted full approval by FDA. By 2009, however, PMOs and 2'-OMePSs had been evaluated in clinical trials for DMD and were observed to have favorable safety and efficacy profiles. van Deutekom et al., *New England J. Med.* 2007; 357:2677-86 at 2678 (“an intraexonic 2OMePS antisense oligonucleotide, PRO051”); Kinali et al., *Lancet Neurol.* 2009; 8:918-28 at 918 (“Intramuscular AVI-4658 [a PMO] was safe and induced the expression of dystrophin locally within treated muscle.”); Arechavala-Gomez et al., *Table 1* (listing several in vivo studies evaluating PMOs or 2'-OMePSs.); Arora et al., *Current Pharm. Biotechnol.* 2004; 5:431-39 at Abstract (“PMOs have shown excellent efficiency and safety profile via various routes of administration in multiple animal and human studies.”) The first exon-skipping drug, Sarepta’s eteplirsen (AVI-4658), was conditionally approved in 2016 with the requirement that Sarepta provide data on functional benefit by 2021. Sarepta has not yet provided such data and will not complete its clinical trial until 2024 or later. Bendicksen et al., *Ann Intern Med.* Epub ahead of print Aug. 22, 2023.

96. In fact, as of 2004, only one AON drug had ever been approved by FDA for any purpose. In 1998, Vitravene, was approved for intravitreal injection (into the eye), but is no longer marketed. Vitravene was superseded in the marketplace by small molecule protease inhibitors and combination treatments. Bradley, *Nat. Milestones* 2019, 5:59.

97. The next AON drug, Kynamro, was not approved until 2013. Kynamro likely works in part because the key cells it targets are liver cells, which are believed to be unusually susceptible to uptake of “naked” AONs without any special chemical modification or vehicle to facilitate delivery. Stein, *Oligos & Peptides* 2014, 32(2):4-7 (“Stein 2014”) at 4.

98. The relative paucity of marketed AONs shows that the unpredictability of this technology extends across all possible therapeutic uses for these drugs, and is not limited to exon skipping. Even when a particular AON chemistry is found to be suitable for one application, it may not be suitable for another. Rather, extensive optimization of any AON is required for any type of treatment.

2. Drug delivery as a barrier to effective treatment

99. In 2005, a major barrier to obtaining effective exon skipping *in vivo* was drug delivery. Indeed, for treatment of DMD, the design of the AON must allow effective delivery to skeletal muscle and cardiac muscle, and potentially other tissues. This was a significant technical challenge, as it requires “systemic” delivery throughout the body.

100. The drug delivery challenges associated with exon skipping were well-documented in the scientific literature. For example, a 2001 publication states as follows:

[L]ike all gene therapy protocols, *the universal problem of delivery* needs to be continually reassessed. Systemic delivery rather than intramuscular injection will undoubtedly be required to distribute the [AONs] if any significant clinical efficacy is to be obtained. Further experiments are necessary to address this problem of delivery . . .

Mann et al., PNAS 2001; 98(1):42-47 (“Mann 2001”) at 47 (emphasis added).

101. In 2002, Aartsma-Rus and colleagues published an article in which they stated that “*theoretically*” targeted exon skipping “*may be* therapeutically applicable.” Aartsma-Rus 2002 at S71 (emphasis added.) In 2003, another publication by Aartsma-Rus and colleagues explained that “[o]ur results indicate that, *provided that a suitable means of administration for the AONs is developed*, antisense-induced reading frame correction *will be* a promising therapeutic approach for many DMD patients carrying different deletions and point mutations.” Aartsma-Rus 2003 at 911 (emphasis added).

102. Moreover, in 2004, the Bremmer-Bout and coworkers wrote as follows:

[I]t may be clear that following injections of reasonable (i.e. affordable) doses of pure AON without any delivery compound, the transfer of AONs into the nuclei of myofibers is too poor to allow sufficient levels of skipping. Therefore, various compounds should be evaluated to identify one that allows high delivery efficiencies without provoking significant toxic side effects. We show here that PEI, compared to the SAINT reagent, was more efficient in delivering the AONs into the myofibers and facilitating exon skipping. However, as expected from previous studies [25,26], PEI is toxic and induces extensive fiber degeneration and regeneration, with dose dependent infiltration of cytotoxic and helper T cells.

Bremmer-Bout et al., Mol. Ther. 2004; 10(2):232-40 (“Bremmer-Bout 2004”) at 238.

103. Furthermore, a 2005 publication co-authored by van Ommen states that “[a]dditional significant development will be necessary to improve the delivery aspects of AON before the antisense approach could be regarded as a realistic therapeutic option in DMD.” Muntoni et al., Nueromuscular Disorders 2005; 15:450-57 at 450.

104. Additionally, a 2011 publication, discussing the use of AONs for treating cancer, states that: “[d]espite huge expenditure on a vast array of delivery strategies, carrier molecules, etc., all of which suffer from cost issues, toxicity, poor delivery to tumors, or a combination, *the foremost technologic hurdle blocking clinical progress for therapeutic [AONs] in cancer is delivery.*” Stein & Goel, Clin Cancer Res 2011; 17(20):6369-72 at 6371 (emphasis added). These hurdles similarly exist for using AONs designed to treat DMD.

105. Also, in 2013, I authored a review article characterizing AON drug delivery for treatment of DMD as a “significant and ever-present challenge,” stating as follows:

One of the most *significant and ever-present challenges facing development of any new therapeutic agent is that of drug delivery.* This holds just as true for oligonucleotide-based therapies and indeed even the most efficient AON in vitro can only ever be as good as its in vivo delivery system. *In DMD, the primary therapeutic target is skeletal muscle. This itself is no small task,* since skeletal muscle typically makes up between 30 and 40% of total body mass. Added to this, BMD patients with in-frame deletions comparable to those that would result from exon skipping treatments currently in development (e.g. exons 51, 53 and 45–55) have been found to express at least 40% of the dystrophin protein levels expressed

by controls and so AON delivery must hope to aim for similar levels of restoration.

Douglas & Wood 2013 at 179 (emphasis added and citations omitted.) DMD treatments must be able to reach cardiac muscle as well as skeletal muscle, but cardiac muscle has proven resistant to AON uptake. Douglas & Wood 2013 at 179.

106. Additionally, a review published in April 2014 states that “an improved understanding of the *in vivo* barriers to oligo delivery” is needed before large scale clinical successes can be obtained. Stein 2014 at 7.

107. AONs with different chemical backbones have very different issues in terms of drug delivery. PS AONs are highly negatively charged and thus hydrophilic, yet must cross hydrophobic lipid bilayers to enter cells. Stein 2014 at 4. For many years, researchers explored the use of carriers such as cationic lipids or other types of charge-neutralizing cationic polymers to facilitate cellular entry. While many of these compounds demonstrate efficacy *in vitro*, their *in vivo* utility is hampered by low efficacy, cost, and toxicity. Bremmer-Bout 2004 at 238 (quoted above); Stein 2014 at 4.

108. Another challenge with PS AON drug delivery results from their “stickiness,” in that they bind many proteins tightly. These unintended interactions can significantly reduce the amount of AON reaching the nuclei of target tissues. Stein 2014 at 5. In some circumstances, these interactions may facilitate delivery or prolong the circulating half-life of the AON. Geary et al., Adv. Drug Deliv. Rev. 2015; 87:46-51 at 46. The effects of these interactions, however, are unknown without testing.

109. While theoretically one could compensate for inefficient delivery by increasing the dose administered, human clinical trials have highlighted several toxicities attributable to the chemical structure of AONs, including AONs with PS backbones. Jason et al., Tox. & Appl.

Pharmacol. 2004; 201:66-83 at 73. These include thrombocytopenia and hyperglycemia, activation of the complement and coagulation cascades, hypotension, and hepatocellular degradation. *Id.* at 74, 78. Thus, for example, Trecovirsen, a PS AON intended to treat HIV, was unsuccessful in clinical trials because of dose limiting toxicities. *Id.* at 76.

3. Lack of animal models

110. Animal models for AON exon skipping validation have limited suitability because AONs are sequence specific and the sequence of a gene often differs between animals and humans. Wu 2011 at 10.

111. This lack of reliable animal models has hindered development of exon skipping therapies for DMD. According to one 2011 paper, “[t]he lack of reliable systems for screening [AON] targeting many human dystrophin exons, especially the lack of animal models for establishing systemic efficacy poses a challenge for [AON] drug development.” Wu 2011 at 2.

112. The *mdx* mouse is one model used to evaluate therapies for DMD. These mice contain a nonsense mutation in exon 23 of the mouse dystrophin mRNA that causes premature termination of translation. Mann 2001 at 42. However, because the mouse and human dystrophin genes have different nucleobase sequences, in many circumstances the *mdx* mouse is not a suitable model to test the efficacy of specific AON sequences against *human* dystrophin.

113. In order to target the human sequence directly, transgenic mice were developed carrying the full-size human *DMD* gene (hDMD). Bremmer-Bout 2004 at 233. This is also a deficient model, however, because the mice do not have a dystrophic phenotype. ‘t Hoen et al., J Biol Chem. 2008; 283(9):5899-907 at 5906. Researchers attempted to overcome this by crossbreeding this transgenic mouse with the *mdx* mouse (hDMD/*mdx*), but expression of human dystrophin compensates for the deficiency in mouse dystrophin. *Id.* at 5906. Thus, this mouse is

also phenotypically normal. Wu 2011 at 10. Because these mice do not display a dystrophic phenotype, the value of assessing drug delivery in these mice is limited because delivery to healthy tissue differs from delivery to dystrophic tissue. As stated in one recent paper, “[t]his has prevented the animal model from being effective for testing AOs . . .” Wu 2011 at 2.

114. Even beyond the delivery issues, the *mdx* mouse and hDMD mouse are limited because exon skipping is assessed in a background where the spliceosome and other key participants in the splicing process are all encoded by mouse genes rather than human genes.

115. There are numerous parameters to optimize to obtain therapeutic exon skipping in humans. These include the choice of delivery compound (efficient delivery versus toxicity); oligochemistry (interaction with delivery compounds, affinity for target RNA sequence, intracellular stability, and degree of toxicity or immunogenicity); and administration method (intramuscular versus systemic). Bremmer-Bout 2004 at 238.

116. Unfortunately, the cell culture and animal studies available to date are a poor proxy for human clinical trials. As stated in one 2010 publication, “predicting the amount of skipping needed *in vitro* for an AO to be therapeutic in a patient is impossible; the efficiency of exon skipping is likely to differ from patient to patient and mutation to mutation, and the levels of dystrophin protein restoration will depend on the quality of the muscle itself when a clinical treatment is started.” Popplewell et al., *Neuromuscular Disorders* 2010; 20(2):102-10 at 109.

V. INVENTIONS IN THE UWA PATENTS

A. Overview of the UWA Patents

117. U.S. Patent No. 9,994,851 (“the ’851 Patent”) issued from U.S. App. No. 15/705,172 (filed on September 14, 2017) on June 12, 2018 and claims priority to U.S. App. No. 15/274,772 (filed Sep. 23, 2016), U.S. App. No. 14/740,097 (filed Jun. 15, 2015), U.S. App. No. 13/741,150

(filed Jan. 14, 2013), U.S. App. No. 13/168,857 (filed Jun. 24, 2011), U.S. App. No. 12/837,359 (filed Jul. 15, 2010), U.S. App. No. 11/570,691 (filed Jan. 15, 2008), PCT/AU2005/000943 (filed Jun. 28, 2005), and Australian App. No. 2004903474 (filed Jun. 28, 2004). Each of the applications in the '851 Patent was a continuation of its immediate parent. The named inventors of the '851 Patent are Stephen Donald Wilton, Sue Fletcher, and Graham McClorey. The '851 Patent's Abstract provides the following summary:

An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 214.

'851 Patent, at Abstract.

118. The '851 Patent has two claims. Claim 1 recites as follows:

An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

Id. at claim 1. Claim 2 recites as follows:

A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

Id. at claim 2.

119. U.S. Patent No. 10,227,590 (“the ’590 Patent”) issued from U.S. App. No. 16/112,371 (filed on August 24, 2018) on March 12, 2019, which is a continuation of and claims priority to, U.S. App. No. 15/274,772 (filed Sep. 23, 2016), U.S. App. No. 14/740,097 (filed Jun. 15, 2015), U.S. App. No. 13/741,150 (filed Jan. 14, 2013), U.S. App. No. 13/168,857 (filed Jun. 24, 2011), U.S. App. No. 12/837,359 (filed Jul. 15, 2010), U.S. App. No. 11/570,691 (filed Jan. 15, 2008), PCT/AU2005/000943 (filed Jun. 28, 2005), and Australian App. No. 2004903474 (filed Jun. 28, 2004). The named inventors of the ’590 Patent are Stephen Donald Wilton, Sue Fletcher, and Graham McClorey. The ’590 Patent’s Abstract provides the following summary:

An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 214.

’590 Patent, at Abstract.

120. The ’590 Patent has two claims. Claim 1 recites as follows:

An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

Id. at claim 1. Claim 2 recites as follows:

A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

Id. at claim 2.

121. U.S. Patent No. 10,266,827 (“the ’827 Patent”) issued from U.S. App. No. 16/112,453 (filed on August 24, 2018) on April 23, 2019 and is a continuation of, and claims priority to, U.S. App. No. 15/274,772 (filed Sep. 23, 2016), U.S. App. No. 14/740,097 (filed Jun. 15, 2015), U.S. App. No. 13/741,150 (filed Jan. 14, 2013), U.S. App. No. 13/168,857 (filed Jun. 24, 2011), U.S. App. No. 12/837,359 (filed Jul. 15, 2010), U.S. App. No. 11/570,691 (filed Jan. 15, 2008), PCT/AU2005/000943 (filed Jun. 28, 2005), and Australian App. No. 2004903474 (filed Jun. 28, 2004). The named inventors of the ’851 Patent are Stephen Donald Wilton, Sue Fletcher, and Graham McClorey. The ’851 Patent’s Abstract provides the following summary:

An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 214.

’827 Patent, at Abstract.

122. The ’827 Patent has two claims. Claim 1 recites as follows:

A method for treating a patient with Duchenne muscular dystrophy (DMD) in need thereof who has a mutation of the DMD gene that is amenable to exon 53 skipping, comprising administering to the patient an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

Id. at claim 1. Claim 2 depends from claim 1 and recites: “The method of claim 1, wherein the antisense oligonucleotide is administered intravenously.” *Id.* at claim 2.

123. The UWA Patents share a common specification with each other and with the United States priority applications and June 28, 2005 PCT priority application. None of the UWA Patents add any additional substantive content to the United States priority applications and PCT priority application.

124. Because the UWA Patents share a common specification and the claims include many identical requirements, (*see, e.g.*, Table 1 below), I will discuss them collectively unless otherwise noted.

Table 1: Exemplary Claim Comparison

'851 Patent, cl. 1	'590 Patent, cl. 1	'827 Patent, cl. 1
An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, <u>wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69)</u> , wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.	An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.	<u>A method for treating a patient with Duchenne muscular dystrophy (DMD) in need thereof who has a mutation of the DMD gene that is amenable to exon 53 skipping, comprising administering to the patient</u> an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

125. As highlighted above, claim 1 of the '851 Patent includes a limitation that specifies the “the target region” of the AO as being “within annealing site H53A(+23+47) and annealing site H53A(+39+69).” Claim 2 of the '851 Patent claims a pharmaceutical composition comprising

(i) the identical antisense oligonucleotides of the '851 Patent claim 1, and (ii) a pharmaceutically acceptable carrier. Claim 1 of the '590 Patent is directed to an AO having the recited features without specifying the coordinates of the “target region.” Claim 2 of the '590 Patent claims a pharmaceutical composition comprising (i) the identical antisense oligonucleotides of the '590 Patent claim 1, and (ii) a pharmaceutically acceptable carrier. Claim 1 of the '827 Patent is directed to a method of treating DMD by administering the antisense oligonucleotides recited in the '590 Patent claim 1, and claim 2 of the '827 Patent depends on claim 1 and specifies that the antisense oligonucleotide is administered intravenously.

126. I have been informed by counsel that certain terms used in the claims of the UWA Patents have been construed by the Court (shown in the table below). I have applied the plain and ordinary meaning of these terms as would be understood by a POSA at the time of the invention in this area of technology in my analysis.

Term #	Term	Claim	Court-Ordered Construction
[1]	“a base sequence”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “any sequence of bases that is part of the antisense oligonucleotide”
[2]	“a target region”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “a segment of the pre-mRNA”

Term #	Term	Claim	Court-Ordered Construction
[3]	“exon 53 of the human dystrophin pre-mRNA”	’851 Patent, claims 1 and 2 ’590 Patent, claims 1 and 2 ’827 Patent, claim 1	Plain and ordinary meaning, which means “the pre-mRNA transcribed from exon 53 of the human dystrophin gene”
[4]	“the target region is within anneal site H53A(+23+47) and annealing site H53A(+39+69)”	’851 Patent, claims 1 and 2	“the target region is within nucleotides +23 to +69 of exon 53 of the human dystrophin pre-mRNA
[5]	“in which uracil bases are thymine bases”	’851 Patent, claims 1 and 2 ’590 Patent, claims 1 and 2 ’827 Patent, claim 1	“the antisense oligonucleotide has thymine bases instead of uracil bases”

B. Exon 53 AONs Disclosed in the UWA Patents

127. The UWA Patents’ specification discloses an invention that “relate[] to novel antisense compounds and compositions suitable for facilitating exon skipping” and “methods for inducing exon skipping using the antisense compounds” ’851 Patent 1:40-42.

128. The inventors disclose 202 AONs for inducing exon skipping in one of exons 3 to 8, 10 to 15, 19 to 40, 42 to 44, 46, 47, and 50 to 53 of the human dystrophin pre-mRNA. ’851 Patent 4:46-49; 7-19. The AOs are identified using a defined nomenclature system of “H#A/D(x:y)” where “the first letter designates the species (e.g., H: human, M: [Murine], C: canine). ‘A/D’ indicates acceptor or donor splice site at the beginning or end of the exon, respectively, (x y) indicates the (x y) represents the annealing coordinates where ‘–’ or ‘+’ indicate intronic or exonic sequences respectively.” ’851 Patent 22:47-55. For example, SEQ ID NO: 191, which is disclosed as H53A(+45+69), anneals to Human Exon 53 at nucleotides 45 through 69 from the start of the exon.

129. Of the 211 AONs disclosed in the UWA Patents’ specification, 12 are directed at exon 53. ’851 Patent 64:46-50. The UWA Patent states that these 12 AONs were tested by

transfecting normal primary myoblasts with 2'OMe oligonucleotides, allowing the cells to grow for 24 hours, and using reverse transcriptase amplification (RT-PCR) to study the targeted regions of the dystrophin pre-mRNA or induced exonic re-arrangements. '851 Patent 32:31-60. The specification provides no other information on experimental parameters, including those critical to assessing the data, such as whether any controls were included or what concentrations were tested.

130. The data concerning exon 53 AONs in the UWA Patents' specification is sparse and inconsistent. Table 2 below provides a summary of the AONs disclosed in the UWA Patents and the inventors' report on their experimental results:

Table 2: UWA Patents Experimental Results

SEQ ID NO.	AON	Length	Ability to Induce Exon Skipping
191	H53A(+45+69)	25	Faint skipping at 50 nM
192	H53A(+39+62)	24	Faint skipping at 50 nM
193	H53A(+39+69)	31	Strong skipping to 50 nM
194	H53D(+14-07)	21	Very faint skipping to 50 nM
195	H53A(+23+47)	25	Very faint skipping to 50 nM
196	H53A(+150+176)	27	Very faint skipping to 50 nM
197	H53D(+20-05)	25	Not made yet
198	H53D(+09-18)	27	Faint at 600 nM
199	H53A(-12+10)	22	No Skipping
200	H53A(-07+18)	25	No Skipping
201	H53A(+07+26)	20	No Skipping
202	H53A(+124+145)	22	No Skipping

Adapted from '851 Patent Table 39 and SEQ ID Nos: 191-202.

131. In addition to the results reported in Table 39 of the specification, the inventors stated that these AONs "showed varying ability to induce exon 53 skipping," and that H53A(+39+69) "induced the strongest exon 53 skipping." '851 Patent 64:48-50. Figure 22 of the UWA Patents provides the only objective data concerning exon 53 skipping. It is a gel showing, *inter alia*, the exon 53 skipping using H53A(+39+69):

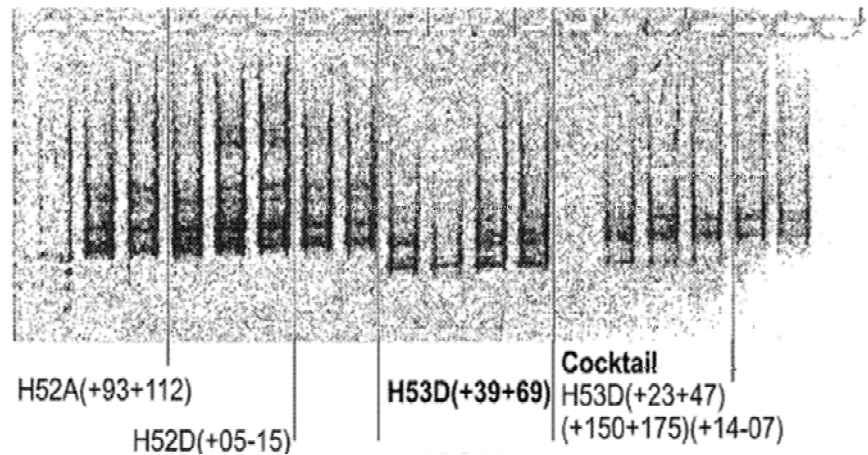


Figure 22 provides little additional insight into what the inventors had discovered about exon 53 AONs generally, or H53A(+39+69)² specifically, as of the priority date. The specification states that Figure 22 shows that H53A(+39+69) “was able to induce exon 53 skipping at **5**, 100, 300 and 600 nM.” ’851 Patent 64:40-41 (emphasis added). However, Table 39 of the specification reports that H53A(+39+69) induced “strong to **50** nM.” ’851 Patent 65 (emphasis added). It is unclear whether this discrepancy is a typographical error or describing different results. This question cannot be resolved by reviewing Figure 22 because the lanes for H53D(+39+69) [sic] are not labeled with the concentration tested.

C. Disclosures Concerning AON Annealing Sites

132. Consistent with my description above that one cannot predict *a priori* whether an AON with a given sequence can induce exon skipping, the inventors explain that when designing AONs, although “choice of target selection” is a critical factor in the efficacy of an AON, “[s]imply designing antisense molecules to target regions of pre-mRNA presumed to be involved in splicing is no guarantee of inducing efficient and specific exon skipping.” ’851 Patent 4:30-35.

² Figure 22 includes experimental results of exon skipping with “H53D(+39+69).” Based on my review of the specification as a whole, I have assumed that this is a typographical error and that Figure 22 shows results from testing H53A(+39+69).

133. The UWA Patents' specification explains that the "inventors [] discovered that there does not appear to be any standard motif that can be blocked or masked by antisense molecules to redirect splicing." '851 Patent 24:4-6. This is consistent with the inventors' note that their "[a]ttempts . . . to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies." '851 Patent 32:15-21.

134. As the inventors stated, H53A(+39+69) induced the strongest skipping of exon 53. Shortened versions of H53A(+39+69), namely H53A(+39+62), H53A(+45+69), were also reported to induce "faint" exon 53 skipping at 50 nM. '851 Patent, Table 39. The other AONs showed varying ability to induce skipping of exon 53 without any clear pattern showing a region outside of H53A(+39+69) that would be an effective target region for AONs to induce exon 53 skipping.

135. Consistent with my earlier discussion, the inventors acknowledged in the UWA Patents' specification that an AON's ability to induce skipping can vary widely with even small changes in the annealing site. For example, AONs designed with overlapping sequences to an AON that had been identified as effective in induce skipping were unable to consistently induce exon skipping. '851 Patent 24:6-12. ("In some exons, such as mouse dystrophin exon 23, the donor splice site was the most amenable to target to re-direct skipping of that exon. It should be noted that designing and testing a series of exon 23 specific antisense molecules to anneal to overlapping regions of the donor splice site showed considerable variation in the efficacy of induced exon skipping.").

136. These findings are further supported by the results of AONs targeting other exons in the UWA Patents’ specification. For example, Table 37 of the UWA Patent reports that H51D(+16-07) induces skipping at 300 nM, while H51D(+08-17) does not induce skipping despite an overlap of 15 bases with H51D(+16-07). In contrast, Table 2 of the UWA Patent reports that H8A(-06+18), H8A(-03+18), H8A(-07+18), and H8A(-06+14) each induces exon skipping at concentrations between 20 nM and 300 nM.

Table 3. Selected Results of Exons 8 and 51 AONs

Target	Sequence	Ability to Induce Exon Skipping
H51D(+16-07)	CUCAUACCUUCUGCUUGAUGAUC	Skipping at 300 nM
H51D(+08-17)	AUCAUUUUUCUCAUACCUUCUGCU	No Skipping
H8A(-06+18)	GAUAGGUGGUAUCAACAUCUGUAA	Very strong to 20 nM
H8A(-03+18)	GAUAGGUGGUAUCAACAUCUG	Very strong to 40 nM
H8A(-07+18)	GAUAGGUGGUAUCAACAUCUGUAAG	Strong to 40 nM
H8A(-06+14)	GGUGGUAUCAACAUCUGUAA	Skipping to 300 nM

Adapted from ’851 Patent Tables 2, 37. Thus, it is not possible to predict whether two or more AONs having overlapping bases will uniformly induce exon skipping merely as a result of the overlapping bases.

D. Disclosures Concerning AON Length

137. The UWA Patents’ specification states that the length of the AONs “may vary” and “generally” will be from 10 nucleotides to 50 nucleotides and “preferably between 17 to 30 nucleotides.” ’851 Patent 25:61-26:3. Nevertheless, the inventors explain that they “discovered that size or length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules.” ’851 Patent 23:60-63. They suggest that optimal exon length may be exon-specific—for exon 19 AONs “as short as 12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides” while for murine exon 23, AONs

“only 17 residues long were able to induce more efficient skipping than another overlapping compound of 25 nucleotides.” ’851 Patent 23:63-24:3.

138. The AONs targeting exon 53 disclosed in the UWA Patents’ specification range from 20 to 31 bases. ’851 Patent 65. Yet, many of these AONs were unable to induce skipping. Because the inventors tested a limited number AONs, it is not possible to determine whether length, annealing site, or a combination thereof affected the AONs efficacy.

139. The unpredictability of AON length on exon skipping activity is further supported by the results of AONs targeting other exons in the UWA Patents’ specification. For example, as shown in Table 4, the UWA Patents’ specification provides experimental results for AONs of 20 to 31 bases targeting the region (-07+25) of human dystrophin exon 16:

Table 4. Selected Results of Exon 16 AONs

Target	Sequence	Length	Ability to Induce Exon Skipping
(-06+25)	UCUUUUCUAGAUCCGCUUUUAAAACCUGUUA	31	Skipping at 5 nM
(-06+19)	CUAGAUCCGCUUUUAAAACCUGUUA	25	Skipping at 25 nM
(-07+19)	CUAGAUCCGCUUUUAAAACCUGUUA	26	No skipping
(-07+13)	CCGCUUUUAAAACCUGUUA	20	No skipping

Adapted from ’851 Patent Table 14.

140. These results show that AONs of varying lengths targeting the same region cannot uniformly induce exon skipping. While a 31mer and a 25mer targeting exon region (-07+25) induced exon skipping, a 26mer targeting this same region did not induce any exon skipping.

141. In contrast, other results in the UWA Patents’ specification show that, for some exons and/or specific regions of exons, AONs of varying lengths targeting the same region are able to induce exon skipping. For example, as shown in Table 5, the UWA Patents’ specification provides experimental results for AONs of 20 to 30 bases targeting the region (+11+40) of human dystrophin exon 4:

Table 5. Selected Results of Exon 4 AONs

Target	Sequence	Length	Ability to Induce Exon Skipping
(+13+32)	GCAUGAACUCUUGUGGAUCC	20	Skipping at 20 nM
(+11+40)	UGUUCAGGGCAUGAACUCUUGUGGAUCCUU	30	Skipping at 20 nM

Adapted from '851 Patent Table 5. Both 20mer and 30mer AONs targeting the region (+11+40) of human dystrophin exon 4 were able to induce exon skipping. Thus, consistent with my description above, the results reported in the UWA Patents show that it is not possible to predict how the length of AON targeting a specific exon will affect its ability to induce exon skipping activity based on results from AONs targeting different exons.

E. The Inventors of the UWA Patents Did Not Have a Definite and Permanent Idea of the Complete and Operative Invention Claimed in the UWA Patents

142. The potentially conflicting description of the H53A(+39+69) results and ambiguous Figure 22 creates uncertainty as to what the inventors had discovered about that AON, and, by extension, what they recognized and appreciated about exon 53-skipping AONs beyond the subjective results provided for the 12 AONs in Table 39.

143. It is my opinion that the inventors of the UWA Patents had, at most, formed a definite and permanent idea that certain specific AONs disclosed by sequence in the UWA Patents' specification induce exon 53 skipping, consistent with the originally-filed claims of the UWA Patents ("What is claimed is: 1. An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 202."). More specifically, at the time the inventors filed the applications leading to the UWA Patents, they had, at most, recognized that H53A(+45+69), H53A(+39+62), H53A(+39+69), H53D(+14-07), H53A(+23+47), H53A(+150+176), and H53D(+09-18) induced some level of exon skipping. *See* Table 39.

144. In my opinion, based on my review of the UWA Patents' specification and the unpredictable nature of the field of AONs, the inventors had not invented any exon 53 skipping AONs beyond those they tested and reported to induce skipping. In particular, it is my opinion that the UWA Patents' specification do not reflect any recognition or appreciation by the inventors of a target region spanning +23 to +69 of exon 53. In my opinion, the UWA Patents' specification do not reflect invention of AONs of 20 to 31 bases that induce exon 53 skipping or a genus of antisense oligonucleotides complementary to 12 bases of H53A(+23+47) by the inventors.

145. More specifically, it is my opinion that the inventors of the UWA Patents had not formed a definite and permanent idea of a range of AON lengths that would induce exon 53 skipping. As the inventors acknowledge in their UWA Patents' specification, that the "length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules." '851 Patent 23:60-63. As I discussed above, the data reported in the UWA Patents' specification shows that AONs of varying the length were not able to consistently induce exon skipping. Moreover, it is not possible to extrapolate results concerning a suitable length or a range of suitable lengths from AONs targeting a different exon to AONs targeting exon 53. Thus, although the UWA Patents' specification states that the length of the AONs "may vary" and "generally" will be from 10 nucleotides to 50 nucleotides" ('851 Patent 25:65-67), identifying AONs of lengths from 10 to 50 nucleotides which induce exon skipping was merely a general goal or research plan the inventors hoped to pursue to identify effective AONs. With respect to exon 53, the information presented in the UWA Patents suggests that longer AONs may be more suitable than shorter ones. H53A(+39+69) is a 31mer, the longest exon 53 AON tested. The other, shorter exon 53 AONs were reported to induce at most faint skipping at 50 nM, even the two that overlapped with H53A(+39+69). Table 39. There is no data in the UWA Patents that indicates

the inventors had formed a definite and permanent idea that AONs as short as 20 bp would be capable of inducing exon 53 skipping.

146. It is my opinion that the inventors of the UWA Patents had not formed a definite and permanent idea that AONs which are complementary to consecutive bases of a target region within annealing site H53A(+23+47) and annealing site H53A(+39+69) would induce exon skipping. The inventors identified only four AONs complementary to consecutive bases within these annealing sites: H53A(+45+69), H53A(+39+62), H53A(+39+69), and H53A(+23+47). Three of these AONs are within the (+39+69) region. Yet only one was reported to induce more than faint skipping at 50 nM as a single AON: H53A(+39+69). Table 39.

147. As the inventors acknowledged, “identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies.” ’851 Patent 32:18-21. The data reported in the UWA Patents’ specification shows even when an AON overlaps with an AON known to induce exon skipping, it is not predictable as to whether the overlapping AON will induce exon skipping. The inventors had not appreciated that all AONs designed to be complementary to bases within annealing site H53A(+23+47) and annealing site H53A(+39+69) would induce skipping of exon 53. Indeed, the data suggests otherwise—only AONs within the annealing site H53A(+39+69) induced “strong skipping,” while H53A(+23+47) showed only “very faint skipping to 50 nM.”

148. To draw the conclusion that AONs targeting the region (+23+69) of exon 53 would induce skipping, the inventors would have had to test a large number of AONs within this region due to the unpredictable nature of the field. Yet, they tested only four, three of which were within the sub-region of (+39+69) of exon 53. Accordingly, identifying AONs which are complementary to consecutive bases within annealing site H53A(+23+47) and annealing site H53A(+39+69) and

which induce exon skipping is merely a general goal or research plan the inventors hoped to pursue to identify additional or more effective exon 53 skipping AONs.

149. It is my opinion that the inventors of the UWA Patents had not formed a definite and permanent idea that AONs comprising at least 12 consecutive bases of H53A(+23+47) (i.e., SEQ ID NO: 195) would induce exon skipping. The inventors had identified only a single AON comprising at least 12 consecutive bases of H53A(+23+47): H53A(+23+47) itself. Thus, based on the UWA Patents' specification, it is unclear how the inventors arrived at claims directed to an AON comprising at least 12 consecutive bases of H53A(+23+47). The UWA Patents' specification does not include *any* data showing which 12 consecutive bases of H53A(+23+47) are necessary or sufficient to arrive at an AON that induces skipping of exon 53, or that AONs shorter than 31 bases are capable of inducing more than faint skipping of exon 53. As I described earlier, the data reported in the UWA Patents' specification shows even when a shorter AON overlaps with a longer AON known to induce exon skipping, it is not predictable as to whether the shorter, overlapping AON will induce exon skipping.

VI. RESERVATION OF RIGHTS AND TRIAL EXHIBITS

150. I reserve the right to amend or supplement my opinions once I review any new/additional information and/or other new/additional documents or information subsequently produced by Sarepta, UWA, or any other party, including via opening expert reports of Sarepta's and UWA's experts.

151. At trial I may use visual aids and demonstratives to show the bases for my opinions, such as photographs, drawings, excerpts from documents and materials I considered, videos, and animations. I reserve the right to utilize these at trial.

Exhibit 17

IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE

NIPPON SHINYAKU CO., LTD.,
Plaintiff,

v.

SAREPTA THERAPEUTICS, INC.,
Defendant.

SAREPTA THERAPEUTICS, INC. and
THE UNIVERSITY OF WESTERN
AUSTRALIA, Defendant and Counter-
Plaintiff

v.

NIPPON SHINYAKU CO., LTD. and
NS PHARMA, INC., Plaintiff and
Counter-Defendants.

C.A. No. 21-1015 (JH)

SUPPLEMENTAL REPLY EXPERT REPORT OF DR. MICHELLE L. HASTINGS
REGARDING INVALIDITY OF THE UWA PATENTS

September 4, 2024



Michelle L. Hastings, Ph.D.

“to Watson-Crick base pair” with pre-mRNA, “*including* those set forth in” the specification).¹

- Dr. Dowdy agrees that “5’ and 3’-end modifications . . . are distinct modifications that could be added” to claimed ASOs. *Id.* ¶ 44.
- Dr. Dowdy does not dispute that the UWA Patents’ specification discloses “at least nineteen different options for chemical moieties.” *See id.* ¶¶ 43-49 (acknowledging my opinion without challenging it).
- Dr. Dowdy does not dispute my conservatively simplified calculation establishing that at least 30,000 chemically-distinct ASO candidates meet the claims’ structural limitations.
- Dr. Dowdy does not dispute my formula for calculating unique combinations of base modifications for ASOs (and even applies it himself in forming his opinions). *Id.* ¶ 40.

13. Thus, despite other disagreements Dr. Dowdy and I may have, the numerical estimates I provide for the number of chemically-distinct ASO candidates that meet the claims’ structural limitations—estimates that employ the number of nucleobase modifications and chemical moieties stated in the UWA Patents’ specification—stand unchallenged. *See Hastings Suppl. Rpt.* ¶¶ 75-78, 81. As I explained, these estimates are conservatively low, as they (1) treat “classes” of chemical modifications or moieties as if they were a single option, and not a category of options; and (2) do not account for variations in inter-subunit linkages.²

14. Dr. Dowdy offers two overarching critiques regarding my analysis of the “full scope” of the claimed genus. First, Dr. Dowdy mischaracterizes my detailed calculation of the number of claimed ASO possibilities resulting from nucleobase modifications, 5’ and 3’ chemical moieties, and inter-subunit linkage variation as being an “afterthought,” and inaccurately suggests that I never contemplated how these variations might affect the claims’ scope while issuing my original opinions. *See, e.g., Dowdy Suppl. Rebuttal* ¶¶ 29, 37, 47. As he knows, however, I now

¹ Emphasis is added throughout unless noted otherwise.

² In other words, these estimates apply even if the claims did preclude variation in inter-subunit linkages.

provide a revised analysis because the Court changed how the claims must be interpreted after I issued my original reports, not because my opinions themselves were in any way deficient.

15. Under the Court's original construction, claimed ASOs could have mismatches. And because Sarepta and UWA provided a mathematical formula during prosecution for calculating ASO possibilities where mismatches are possible, applying that formula was the most straightforward way to estimate the number of ASO candidates. Upon doing so, it became clear that further calculations were unnecessary to demonstrate that the claims' scope was, in fact, vast, encompassing up to trillions of ASOs.³ Nevertheless, as part of offering my original opinions, I specifically contemplated that these chemical variations (1) were disclosed in "the specification of the UWA Patent"; (2) would each "result in chemically distinct AOs"; and (3) would "increase the number of candidates" by at least "a few fold." Hastings Reply Rpt., ¶ 14, n. 2; *see also* Hastings Op. Rpt., ¶ 46 (noting that the "open ended" claims "do not exclude additional unrecited elements"), ¶ 48 (noting that my calculation conservatively "[a]ssum[ed]" only the four naturally occurring nucleobases"). I have proceeded to numerically calculate those figures again, now in a more precise manner focused on the scope of the claims as encompassing only ASOs with 100% complementarity, because the Court has revised its interpretation of the claims to exclude ASOs with mismatches (such that the Sarepta/UWA formula I initially employed in my numerical estimates is no longer directly applicable).

16. As his second overarching critique, Dr. Dowdy argues that the scope of the claims is not "vast" because a POSA "would have focused" on unmodified ASO candidates. *See, e.g.*, Dowdy Suppl. Rebuttal, ¶ 28, n. 4 (intersubunit linkages), ¶¶ 32-33 (nucleobase modifications), ¶

³ *See, e.g.*, Hastings Opening, ¶ 48 (identifying "upwards of 10¹⁴ ASO candidates from nucleobase mismatches"); Hastings Reply, ¶¶ 18-19 (limiting to one or two mismatches still produced tens or hundreds of thousands of ASO candidates).

44 (chemical moieties). This is a non-sequitur. Even assuming Dr. Dowdy was correct about a supposed preference for prioritizing research onto unmodified AO candidates, such a preference would not mean that ASO species with chemical modifications ceased to exist. Modified ASO candidates that meet each-and-every structural claim limitation exist, regardless of whether they rank high or low on a POSA's "to-do list." Any reliable estimation of claim scope must therefore account for them, as I do.

17. Put simply, Dr. Dowdy's rhetorical tack accusing me of "artificially inflat[ing]" the claims' scope is unfounded. *See* Dowdy Suppl. Rebuttal ¶ 14. The written description and enablement inquiries require a POSA to examine the *specification*, and that is where my opinions are grounded. Here, the UWA Patents' specification describes permissible chemical modifications a POSA could employ with ASOs, and those are the modifications I rely upon in opining that the claims' scope is vast. If anything, Dr. Dowdy's attempt to justify why a POSA would disregard those specification disclosures is presumptively an attempt to artificially narrow the genus.

a. Inter-Subunit Linkages

18. In sum, Dr. Dowdy argues that the claimed "morpholino" ASOs are only "phosphorodiamidate morpholino oligomers," and argues that the consideration of four articles during prosecution (Summerton (1997), Heasman (2002), and Aartsma-Rus (2004)) reflects that narrow meaning. *See* Dowdy Suppl. Rebuttal ¶¶ 22-28. I disagree.

19. Summerton (1997) describes variability in morpholino inter-subunit linkages:

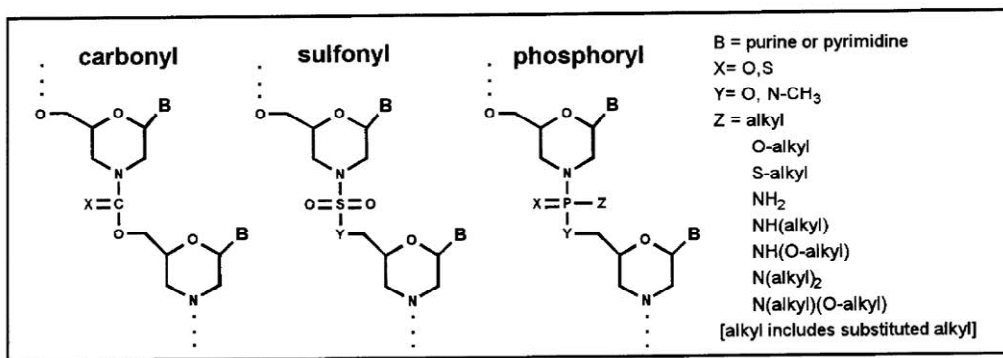


FIG. 4. Intersubunit linkage types for Morpholino oligos.

Summerton (1997) at 189, Fig. 4 (reporting having “assessed a substantial number of intersubunit linkage types, including the carbonyl, sulfonyl, and phosphoryl linkages” and determined that “[m]orpholino oligos containing a number of such linkages provided effective binding”). As Dr. Dowdy’s quotations show, Heasman (2002) and Gebiski (2003) use the general term “morpholino” and cite Summerton 1997’s more general disclosure. Dowdy Suppl. Rebuttal ¶ 23. Aartsma-Rus 2004 uses a more specific term (“morpholino phosphorodiamidate DNA”) than the UWA Patents’ claims use. *Id.* Taken together, these references support my opinion that a POSA would understand “morpholino” to be a generic term that encompasses the “morpholino” chemistries disclosed across these articles (including, but not limited to PMOs).

20. Dr. Dowdy cites the Summerton ’444 Patent (U.S. Patent No. 5,185,444), Dowdy Suppl. Rebuttal, ¶ 28, n. 4, which is a prior art patent that further supports my opinion a POSA would have understood “morpholino” to include more than just PMOs. In Columns 8-10 and Figure 3, the Summerton ’444 Patent describes a variety of chemical inter-subunit linkages that can be employed to synthesize morpholino ASOs.

21. I also disagree with Dr. Dowdy’s characterization that the “United States Patent Office accepted” a purported “definition” during prosecution of U.S. Patent No. 9,024,007. As he quotes, the Examiner described the claimed subject matter using the more general term (“morpholino”), and not the more specific “phosphorodiamidate” language the Applicant later

offered in a response. I do not see any indication that the Examiner adopted the applicant's later description, and the Examiner's earlier statement indicates that the Patent Office understood the term more generally (as Dr. Wood and I apply it).

b. Nucleobase Modifications

22. Dr. Dowdy begins by arguing that “‘morpholino antisense oligomers’ were typically made with naturally occurring bases.” Dowdy Suppl. Rebuttal ¶¶ 32-33. As noted, this misses the point when assessing the scope of the genus. Regardless of whether a POSA sees “practical reasons” to, in some instances, “us[e] only naturally occurring bases,” (*id.* ¶ 33), as of June 28, 2005, a POSA reviewing the specification would have understood that the claims also encompass ASOs having nucleobase modifications. Dr. Dowdy admits that “the Wilton Patents describe nine specific modified bases suitable for the claimed PMOs.” *Id.* ¶ 59. Likewise, he points to the prior art Summerton '444 Patent as “disclosing methods for making morpholino monomers with modified bases.” *Id.* ¶ 161 (citing Summerton '444 at cols. 17-22). And further, he agrees that nucleobase modifications retain sufficient chemistry to allow Watson-Crick base pairing with pre-mRNA in a 100% complementary fashion. *Id.* ¶¶ 34-35.

23. In making his argument, Dr. Dowdy asserts—with no supporting citation—that “as of that time [June 28, 2005], the PMOs tested in clinical trials were made with naturally occurring bases.” *Id.* ¶ 32 (stating he is unaware of “any therapeutic PMO tested in clinical trials before June 28, 2005 that contained any modified bases, let alone multiple modified bases throughout the PMO”). This is misleading. As far as I am aware, there *were no clinical trials* for exon skipping morpholino ASOs at that time. For example, I searched ClinicalTrials.gov for any clinical trial

records using the terms “morpholino” or “PMO,” and the earliest study listed was NCT00159250,⁴ the Phase I / II study for Eteplirsen. The earliest “Study Record” (from September 8, 2005) indicates that no particular ASO had yet been selected for the trial,⁵ and the “Study Start (Actual)” for this trial did not occur until October 26, 2007.⁶

24. Dr. Dowdy next argues that the existence of claimed ASOs with modified nucleobases “do[es] not meaningfully increase” the claims’ scope. *See id.* ¶ 36. But, other than generically noting that modified nucleobases still function according to Watson-Crick principles, Dr. Dowdy cites no support for his argument. He offers no competing estimate of how much these additional “optional modifications” numerically increase the number of chemically-distinct candidate ASOs that meet the claims’ structural limitations beyond the “168” or “330” target regions.

25. And again, Dr. Dowdy’s argument does not logically follow. Although ASOs with and without modified nucleobases engage in Watson-Crick pairing, they nevertheless are chemically distinct ASOs from each other. Thus, that the claims cover both ASOs with and without modified nucleobases means that the genus encompasses far more chemically-distinct

⁴ *Search Results*, CLINICALTRIALS.GOV, <https://clinicaltrials.gov/search?term=morpholino%20OR%20PMO&sort=StudyFirstPostDate&page=2> (last visited Sep. 4, 2024).

⁵ *Safety and Efficacy Study of Antisense Oligonucleotides in Duchenne Muscular Dystrophy*, CLINICALTRIALS.GOV, <https://clinicaltrials.gov/study/NCT00159250?term=morpholino%20OR%20PMO&sort=StudyFirstPostDate&page=2&rank=45&tab=history&a=1#version-content-panel> (last visited Sep. 4, 2024) (making no mention of PMOs, targeting exon 51, eteplirsen, or AVI-4658). The first mention of PMOs, exon 51, and AVI-4658 comes in the February 19, 2007 clinical trial record update.

⁶ *Safety and Efficacy Study of Antisense Oligonucleotides in Duchenne Muscular Dystrophy*, CLINICALTRIALS.GOV, <https://clinicaltrials.gov/study/NCT00159250?term=morpholino%20OR%20PMO&sort=StudyFirstPostDate&page=2&rank=45&a=1> (last visited Sep. 4, 2024).

ASOs than if the genus covered *only* ASOs having natural nucleobases (as my exemplary calculations illustrate). Dr. Dowdy's citation to the NS Patents' more robust list of nucleobase modifications, (Dowdy Suppl. Rebuttal ¶¶ 39-40), only confirms that my calculated estimates—which use the UWA Patents' narrower list—are conservatively low.

26. I also disagree with Dr. Dowdy's attempt to liken the claims of the NS Patents to the UWA Patents. *See* Dowdy Suppl. Rebuttal ¶¶ 38-41. The NS Patents claim far less than the UWA Patents and yet disclose substantially more experimental work on exon 53. Unlike the UWA Patents, all of NS's asserted claims correspond to *one*, exact target region (h53A+36+60) that the NS and NCNP inventors *actually tested* and for which the specification reports quantitative data showing high levels of exon 53 skipping across multiple rounds of testing. *See, e.g.*, '092 Patent, figs. 9, 13, 16-17. Also, unlike the UWA Patents, the NS and NCNP inventors *actually tested* both PMOs and 2'OMe-PS ASOs directed to exon 53, and even comparatively tested PMO activity after making chemical modifications to the PMO. *See, e.g., id.* at fig. 8, tbl. 2 (showing that two PMOs retained much of their activity when modifying their 5' end group). And further unlike the UWA Patents, the NS Patents include claims that require ASOs to have natural nucleobases and/or the exact 5' modifications for which the NS and NCNP inventors had demonstrated results in PMOs. *See, e.g.*, '092 Patent, cls. 2-3; '461 Patent, cls. 1-2; '106 Patent, cls. 1-2; '741 Patent, cls. 2-3, 5-6, 8-9, 11-12; '217 Patent, cls. 1-4.

27. In short, it is one thing to conclude—after having performed comparative testing using different ASO backbones and chemical modifications—that a highly-active ASO directed to the exact same target region would retain exon skipping activity (as the NS and NCNP inventors did). It is quite another to speculate—without *any comparative testing whatsoever*—that the “very faint” activity observed with SEQ ID No: 195 would be preserved when changing the chemical

backbone, substantially lengthening or shortening the ASO, shifting it to share less than half of the original “target region,” *and then also* allowing further chemical modifications to nucleobases and/or the 5’- or 3’- ends (as Dr. Dowdy claims a POSA would in considering the UWA Patents’ claims).

c. Chemical Moieties (5’- and 3’- Modifications)

28. Dr. Dowdy’s core argument is that “end moieties were not understood as key components for inducing exon skipping,” (Dowdy Suppl. Rebuttal ¶¶ 43-47), but this again misses the point when assessing the scope of the genus. Regardless of whether considered a “key component,” as of June 28, 2005, a POSA reviewing the specification would nevertheless have understood that the claims encompass ASOs having chemical moieties. Dr. Dowdy admits that there are “distinct modifications that could be added” to claimed ASOs, (*id.* ¶ 44), and that the specification discloses them, (*id.* ¶ 45). The resulting, chemically-distinct ASOs must therefore be accounted for when assessing the breadth of the claims.

29. Further, I disagree with Dr. Dowdy’s assertion that chemical moieties “are peripheral for inducing exon skipping” and that their “impact [on] properties like solubility or delivery” is “peripheral” or “not essential to the claimed function.” Dowdy Suppl. Rebuttal ¶ 44-45. I discuss this below, in Section V.A.2.C.

d. Contextual Evidence of the Genus’s Size

30. Dr. Dowdy critiques my contextualization of the genus’s vast scope against the exon skipping work POSAs had actually performed (both for exon 53 and generally), arguing that “the numbers of exon 53 targeting ASOs disclosed in prior art references . . . simply reflects different experimental designs used by different research groups,” and that “the comparison should

focus on the capacity to make and test ASOs generally.” Dowdy Suppl. Rebuttal, ¶ 52. But Dr. Dowdy fails to present any evidence that rebuts my conclusions.

31. Regarding exon 53 specifically, Dr. Dowdy does not (and cannot) contest that “[h]istorically, when a group of POSAs—whether an academic institution or company—decided to examine exon 53 skipping, those groups tested relatively few exon 53-directed ASOs.” Hastings Suppl. Opening Rpt. ¶¶ 67-68. He likewise does not (and cannot) contest that “no POSA had reported testing more than a single ‘target region’ with morpholino ASOs at a given time.” *Id.* ¶ 69. The import of this fact is clear—the UWA Patents’ claimed genus would have been, as of June 28, 2005, recognized by any POSA as **unprecedentedly broad** compared to all previously reported exon 53 work. This conclusion is true even if only the 168 “target regions” were considered, and not the far greater number of chemically-distinct ASOs captured by the claims.

32. Dr. Dowdy attempts to excuse this unprecedented breadth by (inaccurately) suggesting that “making and testing **PMOs** directed to 168 or 300 target regions was well within the capacity of a POSA as of June 2005.” Dowdy Suppl. Rebuttal, ¶¶ 53-54. But he fails to cite any example of a POSA before June 2005 having tested anything close to those kinds of numbers of morpholino ASOs.

33. The largest number of morpholino ASOs Dr. Dowdy identifies any POSA testing for **any** purpose before June 28, 2005 are the 56 morpholino ASOs described in Deere (2005) (a mere one-third the number of “target regions” encompassed by the ’851 Patent’s claims), whose authors were all affiliated with AVI BioPharma. *See id.* ¶ 55. The PMOs only ranged from “7 to 20 bases” long, and were used to “inhibit gene expression” (not induce exon skipping). Deere (2005) at Abstract, 249. The PMOs tested varied in activity (luciferase inhibition) based upon both length and positions within the *myc-luc* pre-mRNA transcript. *See, e.g., id.* at figs. 2-4, 6.

None of the PMOs contained any nucleobase modifications, 5' moieties, nor 3' moieties. *See generally id.* at tbl. 1. This fact reinforces my opinion that a POSA would recognize the number of morpholino ASOs encompassed by the claimed genus (even if limited to the 168 target regions) as being of unprecedented breadth.

34. Similarly, although my Opening Supplemental Report identified the 114 ASOs mentioned by Aartsma-Rus (2005), (Hastings Suppl. Opening ¶¶ 70, 73), Dr. Dowdy fails to identify any instance in which a POSA tested any larger number of ASOs—regardless of backbone chemistry—for exon skipping before June 28, 2005.⁷ Further, as I mentioned, these 114 ASOs represent testing only “77 new AONs.” Aartsma-Rus (2005) at Abstract (“We have previously described 37 exon-internal AONs . . .”). The remaining ASOs were previously reported in, *e.g.*, the Van Ommen patent application, which lists certain Aartsma-Rus (2005) co-authors as named inventors. Thus, the 114 ASOs reported by Aartsma-Rus (2005) show that, even after a leading laboratory spent *years* working in the field, it had still tested materially *fewer* than the 168 or 330 “target regions” encompassed by the UWA Patents. This reinforces my opinion that a POSA would recognize the number of ASOs encompassed by the claimed genus (even if limited to the 168 target regions) as being of unprecedented breadth.

35. The post-priority testing that Dr. Dowdy cites—Poplewell (2009), Sazani PCT '586, Wilton Mol. Ther. 2007 and the Prosensa '802 Publication—further supports my opinions. These confirm that testing the breadth of ASOs encompassed by the UWA Patents' claims would have remained unprecedented for years after the priority date. Notably, Dr. Dowdy

⁷ Aartsma-Rus 2005 was not published until December 2005. *See* SRPT-VYDS-02457610 at 613. So, the largest number of ASOs either Dr. Dowdy or I have identified a group of POSAs reporting testing for exon skipping by June 28, 2005 is even less—the 88 ASOs described in Matsuo (just over half as many “target regions” encompassed by the '851 Patent's claims). *See* Dowdy Suppl. Rebuttal, ¶¶ 53-54.

does not (and cannot) contest that Popplewell (2010) characterized testing as few as 66 ASOs as being a “*large* screen[.]” See Hastings Suppl. Opening ¶¶ 70-72.

36. Additionally, the Prosensa ’802 Publication contradicts Dr. Dowdy’s opinions that (1) POSAs recognized his alleged “hot spot”; and (2) that POSAs would not test every exon 53 “target region,” but would instead test “a smaller number of ASOs” across the scope of the claims. The Prosensa ’802 Publication describes testing “[a] series of AONs targeting sequences” within “a continuous nucleotide stretch within exon 53 herein defined as SEQ ID NO 7.” ’802 Publication at 30:22-28, 33:25-27, tbl. 6. That stretch is entirely outside Dr. Dowdy’s alleged “hot spot”—it spans positions 70 to 141 of exon 53. The Prosensa researchers did not merely test a few “target regions” within their region of interest—they iteratively tested every possible 25-mer target region.⁸ See *id.*

37. In short, there is no basis to characterize the scope of the UWA Patents as “narrow.” Dr. Dowdy inappropriately ignores the number of chemically-distinct ASOs having each claims structure and incorrectly evaluates only the number of “target regions” implicated by the claims. Even then, he fails to acknowledge that the work conducted by leading scientists in the field—not just those of *ordinary* skill—paled in relative scope compared to the breadth of ASOs (and even “target regions”) implicated by the UWA Patents’ claims. Dr. Dowdy’s suggestion that POSAs at the time could have “quickly” or “rapidly test[ed] every target region” is belied by the fact that *none* of the contemporaneous publications from scientists in the field report conducting such

⁸ Specifically, SEQ ID No. 358 is 100% complementary to the twenty-five positions farthest 5’ (+70+94), SEQ ID No. 311 is 100% complementary to the twenty-five positions farthest 3’ (+117+141), and the other ASOs are 100% complementary to each other “target region” in between,

extensive experimentation, nor conducting such experimentation in the relatively shorter time periods Dr. Dowdy asserts.⁹

2. The UWA Patents Do Not Disclose any Common Structure-Function Correlation Across the Genus

a. Dr. Dowdy's Identifies Neither a "Common" Structure Nor a "Correlation" to Exon Skipping Disclosed by the Specification

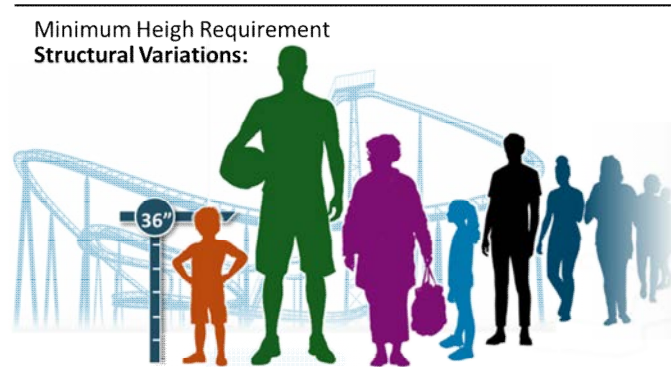
38. I understand that a patentee may satisfy written description for a functional genus by disclosing sufficient structural features common to the members of the genus so that one of skill in the art can visualize or recognize the members of the genus—*i.e.*, to recognize *functional* compounds (members of the genus) amongst the various candidate compounds. I understand that, for written description to be sufficient, a patentee must do more than merely draw a fence around the outer limits of a purported genus, and that drawing such a fence is no substitute for actually demonstrating possession of a variety of materials that constitute the genus.

39. Dr. Dowdy's analysis mischaracterizes the claims' structural "fenceposts" as being structures "common" across the functional members of the claimed genus. *See* Dowdy Suppl. Rebuttal ¶¶ 57-58. In doing so, he fails to identify any structure that is both "common" across members of the genus (each functional ASO) *and* that the specification discloses as being correlated with the claimed function (exon 53 skipping).

40. To illustrate this deficiency, consider a 3-foot (36") minimum height requirement for a rollercoaster. Everyone riding the rollercoaster meets the same "shared" structural criteria of being over 3 feet tall, but that does not mean that they share any common (or even similar) height:

⁹ As discussed above and in my Supplemental Opening Report (¶¶ 70-72), the later-in-time articles that discuss relatively larger numbers of ASOs (*e.g.*, Wilton Mol. Ther. 2007, Aartsma-Rus (2005), Aartsma-Rus (2008)) are retrospectives describing many years' worth of research that had been compiled.

both a kindergartener and NBA player (vastly different “structures” in terms of height) would meet this same criteria.



Thus, to accurately determine whether structural commonalities exist across the functional genus, one cannot solely consider the structural *criteria*. One must also consider variation across the ASOs themselves.

41. Unlike Dr. Dowdy, I considered *both* the claims’ structural criteria and then the structural variety across ASOs meeting those criteria. *See* Hastings Suppl. Opening ¶¶ 114-126. As I explained, the claims’ structural criteria do not result in any “common structures” across ASOs except for (as to the ’851 Patent) a seven-base segment complementary to h53A(+36+42). *See id.* Dr. Dowdy does not (and cannot) contest that the ASOs making up the ’590 and ’827 Patents’ genus need *not* share any “common” sequence of bases. *See* Dowdy Suppl. Rebuttal ¶¶ 66-70 (disputing the relevance of my analysis without challenging this assertion). He likewise does not (and cannot) contest that the only “structure” present across each and every species within the ’851 Patent’s claimed genus is being a morpholino having 100% complementarity to dystrophin pre-mRNA with seven bases complementary to h53A(+36+42) and thymine bases.¹⁰

¹⁰ This analysis explicitly accounts for all structural claim limitations. Dr. Dowdy’s assertion that I am “ignor[ing] other structural features” beyond base sequences is unfounded. *See* Dowdy Suppl. Rebuttal ¶ 69.

See id. (same). Dr. Dowdy does not even contest that the specification “fail[s] to explain how the overlapping region, H53A(+36+42) correlates with exon 53 skipping.” *Id.* ¶ 68 (arguing only that this is “irrelevant” because those seven bases fall within the alleged “hot spot”).

42. Dr. Dowdy’s argument for a structure-function correlation across the claimed genus rests on his purported “hot spot.” *See* Dowdy Suppl. Rebuttal ¶ 61 (arguing that a POSA would expect function “because [candidate PMOs] target the exon 53 hot spot”); *see also id.* ¶¶ 67-69 (critiquing my analysis based upon his belief in the “hot spot”). But while Dr. Dowdy accuses me of “ignor[ing] . . . structural features that are expressly disclosed and claimed,” (*id.* ¶ 69), he does exactly that by attempting to extrapolate “four overlapping ASOs” that are *outside* the claimed genus into a correlation *throughout* the claimed genus, (*id.* ¶ 62). As illustrated below, the four ASOs Dr. Dowdy relies upon each fail to meet two or more claimed structural criteria.

Encompassed Structures	SEQ ID No. 195 (h53A+23+47)	SEQ ID No. 192 (h53A+39+62)	SEQ ID No. 191 (h53A+45+69)	SEQ ID No. 193 (h53A+39+69)
20 bases	NO	NO	NO	NO
21 bases	NO	NO	NO	NO
22 bases	NO	NO	NO	NO
23 bases	NO	NO	NO	NO
24 bases	NO	YES	NO	NO
25 bases	YES	NO	YES	NO
26 bases	NO	NO	NO	NO
27 bases	NO	NO	NO	NO
28 bases	NO	NO	NO	NO
29 bases	NO	NO	NO	NO
30 bases	NO	NO	NO	NO
31 bases	NO	NO	NO	YES
100% Complementary	YES	YES	YES	YES
At least 12 consecutive bases	YES	NO	NO	NO
Thymine bases	NO	NO	NO	NO
Morpholino	NO	NO	NO	NO
Any Nucleobase Modification(s)	NO	NO	NO	NO
Any 5’ or 3’ End Moiety	NO	NO	NO	NO

43. Further, even setting aside the claims’ morpholino and thymine requirements, these four disclosed ASOs still do not reflect a variety of materials that constitute the genus as a whole.

These ASOs use only 3 of the 12 claimed lengths, 1 of the 168 “target regions,” no less than 25 consecutive bases of SEQ ID No. 195 (*i.e.*, none with between 12 and 24 consecutive bases), none of the permitted nucleobase modifications, and none of the permitted 5’ or 3’ chemical moieties. And only one of the four ASOs comprises a portion complementary to the seven-bases spanning h53(+36+42) (SEQ ID No. 195 itself). In my opinion, no POSA would extrapolate this scant (and largely subjective) report of activity to draw the “hot spot” conclusion Dr. Dowdy reaches, particularly given the high levels of unpredictability in the art.

44. Dr. Dowdy’s description of a hypothetical “rational course of experimentation” across the genus also provides a telling contrast from his reliance on the four disclosed ASOs:

[A] POSA could have limited her testing to 20-mer, 25-mer, and 31-mer ASOs as illustrated below. If, as expected, each ASO in the group induces exon 53 skipping, then a POSA would have understood that ASOs of intermediate lengths would also induce exon 53 skipping. With this approach, a POSA could rapidly test every target region with a high degree of confidence.

Dowdy Suppl. Rebuttal ¶ 54. In this hypothetical, Dr. Dowdy argues that a POSA would design experiments to test the outer boundaries (20-mer and 31-mer) and an approximate mid-point (25-mer) of the ASO lengths across the genus, and then use that data to extrapolate inwards to draw conclusions regarding “ASOs of intermediate lengths”—*i.e.*, a POSA would draw conclusions about ASOs *within* the confines of the structural features actually tested in Dr. Dowdy’s hypothetical.¹¹ But with the specification’s data, Dr. Dowdy starts with results for ASOs *outside*

¹¹ Given the high level of unpredictability in the art and the material effect that even small chemical changes can have on exon skipping (as is detailed throughout my reports), I disagree that a POSA would, as of June 28, 2005, have made even this type of extrapolation.

the confines of the claimed structural features and then attempts to extrapolate a correlation outwards to reach conclusions about the claimed subject matter.¹²

45. As an example, Dr. Dowdy highlights the specification's teachings that nucleobase modifications and end modifications are possible, and asserts that a POSA could "visualize PMOs" with these modifications. Dowdy Suppl. Rebuttal ¶¶ 59-60. I agree that a POSA could identify the chemical structures of ASO candidates with such chemical modifications, but that does not mean a POSA could recognize the *functional* members of the genus from amongst those candidates. Dr. Dowdy's only attempt to address these modified ASO candidates is to sweepingly assert that "most candidate PMOs, if not all, would induce exon 53 skipping because they target the exon 53 hot spot . . . regardless of whether they contain modified bases and/or an end modification." *Id.* ¶ 61. As discussed, however, this extrapolation has no basis—the evidence from which he seeks to establish the "hot spot" (and the rest of the specification), lacks any data corresponding to ASOs with nucleobase or end modifications.

46. What Dr. Dowdy argues is akin to an inventor in 1900 claiming that they had invented all two-winged flying machines after observing that birds with two wings fly. While, of course, some machines with that structure (two wings) fly, having two wings alone is not sufficient to reasonably guarantee flying (*e.g.*, flightless birds, failed airplane designs, a bicycle with wings fashioned from bedsheets). The alleged inventor would not have done sufficient work to show others what combinations of materials, sizes, and shapes in two-winged designs would actually allow one to fly. And they certainly would not have shown they had invented the highest-functioning designs (*e.g.*, jet planes). Dr. Dowdy's arguments are also akin to arguing that that a

¹² In fact, Dr. Dowdy has no choice but to draw such an extrapolation if he is to argue that the specification discloses a "structure-function" correlation. The specification includes *no data* corresponding to testing claimed ASOs.

few physical traits (*e.g.*, height, weight, speed) will dictate whether a prospect will actually prove to be a successful pro athlete. While those traits might affect a prospect's eventual success, being a good basketball, football, baseball, or hockey player takes more than just being tall and strong.

b. Dr. Dowdy's Critiques Regarding Unpredictability Are Misplaced

47. As my Supplemental Opening Report details, I disagree with Dr. Dowdy's characterizations of the evidence I rely upon to demonstrate unpredictability in the art. *See generally* Dowdy Suppl. Rebuttal, ¶¶ 71-90. Although I focus my reply here to particular issues, that should not be understood as agreement to Dr. Dowdy's other mischaracterizations.

48. *The Specification's Data*: Dr. Dowdy claims that "it is irrelevant that different ASOs targeting the hot spot induced different levels of exon skipping." *Id.* ¶ 75. This statement is another example of Dr. Dowdy ignoring structural variability across ASOs within the claimed genus. A POSA examining the exon 53 ASOs described by the specification would understand that they are complementary to substantially different "target regions" and have different lengths. As just those two parameters changed, exon skipping reportedly dropped from "Strong skipping," to "Faint skipping," to "Very faint skipping," to "No skipping." *See* '851 Patent, tbl. 39 (describing SEQ ID Nos. 191, 192, 193, 195, 201). This data indicates that exon-skipping changes as "target region" and "length" change, and that such changes may include precipitous drops in exon skipping, including dropping to "No skipping."

49. Notably, because the claims are centered around an ASO reported to have "very faint skipping" (SEQ ID No. 195)—not a highly active ASO—even a minor decrease in exon skipping activity would produce a completely inactive ASO. And yet, the claims not only allow variation along these same parameters (168 or 330 target regions, 20 to 31 bases in length), but also require variation relative to the disclosed ASOs along other parameters (using thymine bases,

morpholino chemistry), and allow further variation along yet other parameters (nucleobase modifications, end modifications). Therefore, as of June 28, 2005, the varying levels of exon-skipping activity reported in the specification would have *confirmed* to a POSA that they could *not* predictably expect exon skipping across the variety of claimed ASOs.

50. Teachings of the Specification and UWA Publications: Dr. Dowdy re-interprets the UWA researchers' statements with hindsight to fit his opinions regarding the "hot spot." See Dowdy Suppl. Rebuttal ¶¶ 72-73, 77-78. Neither the specification nor Wilton Mol. Ther. 2007 mention having "empirically determined" any purported "hot spot" or area of heightened activity for exon 53, much less one spanning bases +23 to +69.

51. Prosecution of the '851 Patent and '007 Interference: Dr. Dowdy mischaracterizes the statements at-issue as solely relating to "skipping of other exons," "skipping of exon 53 with ASOs targeting areas outside the hot spot," and "statements directed to the state of the art prior to the disclosure of the Wilton Patents," and opines that are each "immaterial to the claimed ASOs." Dowdy Suppl. Rebuttal ¶¶ 80-83, 84-89. I disagree.

52. **First**, the predictability of and state of the art in the years leading up to the UWA Patents are highly relevant, as they reflect the knowledge and perspective that the hypothetical POSA would bring to evaluating the UWA Patents' specification.¹³ For example, because a POSA would approach the UWA Patents' specification understanding that the art was highly unpredictable, they would not extrapolate beyond the scant results disclosed therein to untested

¹³ I understand that written description is evaluated from the perspective of a POSA. Similarly, as to enablement, the predictability of and state of the art in the years leading up to the UWA Patents correspond to *Wands* factors.¹³ See Hastings Suppl. Opening Rpt., ¶ 27 (identifying four of the *Wands* factors a "the presence or absence of working examples in the specification," "the amount of direction or guidance presented in the patent specification," "the predictability or unpredictability of the art," and "the state of the prior art").

structures, much less conclude that those scant results established a “hot spot” (especially given the lack of any statement in the specification asserting that such a “hot spot” had been discovered).

53. **Second**, Dr. Dowdy’s attempt to discount reliance on studies involving ASOs from outside his “hot spot” is belied by his own reliance on such studies. Less than ten pages before he asserts that “skipping of other exons” and “ASOs targeting areas outside the hot spot” are “immaterial,” (Dowdy Suppl. Rebuttal ¶ 83), he relies upon the results from Errington (2003) as supposed evidence that “an ASO with 100% complementarity generally induces more robust exon skipping than a corresponding ASO lacking 100% complementarity,” (*id.* ¶ 63). The 2’OMe-PS ASOs described in Errington (2003), however, relate **only** to exons 19 and 23, and three targeted mouse dystrophin pre-mRNA. Errington (2003) at tbl. 1.

54. Likewise, less than five pages after he asserts that “skipping of other exons” and “ASOs targeting areas outside the hot spot” are “immaterial,” (Dowdy Suppl. Rebuttal ¶ 83), he relies upon the results from Gebiski (2003) as supposed evidence that “the exon skipping ability exhibited by a 2’OMePS ASO is generally translatable to a corresponding PMO targeting the same region,” (*id.* ¶ 93). The ASOs described in Gebiski (2003), however, were directed to only one target region of mouse exon 23. Gebiski (2003) at 1803.

55. **Third**, Dr. Dowdy’s critique is irreconcilable with Sarepta’s and UWA’s reliance upon prior art studies and/or studies involving ASOs from outside the “hot spot” in the ’851 Patent’s prosecution.¹⁴ As noted in my Opening Supplemental Report, the obviousness rejection

¹⁴ At least the van Ommen ’952 Publication, Aartsma-Rus (2002), and Aartsma-Rus (2005) reported on an ASO within Dr. Dowdy’s “hot spot,” namely h53AON1, which was an 18mer 100% complementary to positions 45 to 62 of exon 53. Additionally, the Aartsma-Rus (2009) article was a “retrospective analysis” by the University of Leiden researchers (*e.g.*, Dr. Aartsma-Rus and Dr. van Deutekom) reporting on the ASOs they had “previously designed and evaluated for splice modulation of the dystrophin transcript.” Aartsma-Rus (2009) at 548.

involved the same claim language that ultimately issued and centered on an 18-mer ASO complementary to positions h53A(+45+62). Hastings Suppl. Opening Rpt. ¶ 99. Sarepta and UWA cited prior art studies and studies involving ASOs from outside the “hot spot” to show that this ASO—an ASO *within* Dr. Dowdy’s “hot spot”—could not be predictably modified. *Id.* ¶¶ 99-103. Further, Sarepta and UWA made clear that, contrary to Dr. Dowdy’s opinions, this “unpredictability” was not resolved by the UWA Patents, but instead “*continued beyond 2005.*”¹⁵ *Id.* ¶ 102. If these studies were, as Dr. Dowdy claims, wholly irrelevant to the claimed subject matter and did not evidence continued unpredictability “beyond 2005,” Sarepta and UWA had no basis to make these arguments to the Patent Office.

56. **Fourth**, Dr. Dowdy’s critique is irreconcilable with Sarepta’s and UWA’s reliance upon prior art studies and/or studies involving ASOs outside exon 53 in the ’007 Interference.¹⁶ Dr. Dowdy does not contest that the claimed subject matter at-issue in the ’007 Interference related to exon 53 ASOs that induced skipping. In fact, he highlights that the AZL patent claims required ASOs “comprising at least 15 bases of the sequence [SEQ ID NO: 29],” (Dowdy Suppl. Rebuttal ¶ 85), which corresponds to “h53AON1”—the same prior art ASO complementary to positions h53A(+45+62) that the Patent Office would later cite in its obviousness rejection of the ’851 Patent, (*see* ’952 Publication, tbl. 2), and which is undisputedly complementary to a target region located within Dowdy’s purported “hot spot.” In other words, just as Dr. Dowdy claims that the SEQ ID NO: 195 limitations “anchor[]” the UWA Patents’ claims “to the hot spot,” (Dowdy Suppl. Rebuttal ¶ 69), so too were the AZL claims at-issue anchored to Dr. Dowdy’s “hot spot.”

¹⁵ Dr. Dowdy does not attempt to offer any explanation for this statement.

¹⁶ The van Ommen ’952 Publication’s underlying patent application, Aartsma-Rus (2002), Aartsma-Rus (2005) and Aartsma-Rus (2009) were also discussed during in the ’007 Interference.

57. Dr. Dowdy attempts to distinguish the claim scope, (*see id.* ¶¶ 85-89), but that does nothing to rebut my point. I am not arguing that the UWA Patents must be found invalid just because similar AZL claims were found invalid on similar grounds. Rather, I am looking to how Sarepta and UWA characterized the state of art regarding exon 53 skipping during their negotiations with and arguments to the Patent Office and considering those facts in my independent analysis of the UWA Patents. As discussed in my Opening Supplemental Report, Sarepta and UWA explicitly stated that “[s]ubsequent experience” for exon 53 from after “the filing dates of the “competing applications” (*i.e.*, **after 2005**) “revealed that operative sequences are actually highly unpredictable.” Hastings Suppl. Opening Rpt. ¶¶ 104. They likewise stated that “[e]xon skipping of dystrophin pre-mRNA *was* a nascent and highly unpredictable technology . . . **and remains so today** [in 2014].” *Id.* ¶ 105.

58. In any event, Dr. Dowdy’s attempts to distinguish the AZL claims are inconsistent with other opinions he offers. For example, Dr. Dowdy opines that the “’007 Interference claims “were not limited to ASOs with 100% complementarity.” Dowdy Suppl. Rpt. ¶ 86. But the ’007 Interference claims recite “[a]n isolated **antisense oligonucleotide**,” (UWA Motion 1 (Nov. 18, 2024) at 10), and Dr. Dowdy has opined that “the term ‘antisense oligonucleotide’ refers to a short string of nucleotides (‘oligonucleotide’) that is **complementary** (‘antisense’) **to a discrete target region** present in the pre-mRNA transcript” and allow only 1-2 mismatches or insertions, (Dowdy Rebuttal Rpt. ¶ 35. Dr. Dowdy also points to the AZL claims’ allowance “for multiple different backbone chemistries and ribose ring modifications” as a purported difference, (Dowdy Suppl. Rpt. ¶ 86), while elsewhere opining that having a different chemical backbone or chemical modifications is “peripheral” or “irrelevant,” (*see, e.g., id.* at ¶¶ 35, 42, 44-45, 48, 93-96, 99-100, 133). Dr. Dowdy cannot reconcile these contrary opinions.

59. Regardless, as I mentioned, Sarepta and UWA not only prevailed in persuading the PTAB of their views regarding the high unpredictability of exon 53 skipping, and they cited that finding to the Patent Office during prosecution of the '851 Patent. Hastings Suppl. Opening Rpt. ¶ 107. If the '007 Interference were, as Dr. Dowdy claims, wholly irrelevant to the claimed subject matter and did not evidence continued unpredictability beyond 2005, Sarepta and UWA had no basis to make that argument to the Patent Office. Of course, I agree with Sarepta and UWA's original statements to the Patent Office that exon skipping was unpredictable in June 2005 and remained so in 2014 (as does Dr. Wood). It is only Dr. Dowdy who says otherwise.

c. A POSA Would Need to Test ASO Candidates, Not Just "Target Regions"

60. Throughout his report, Dr. Dowdy suggests that a POSA would not need to account for the full structural variability encompassed by the claims, and only contemplates testing the 168 or 330 target regions encompassed by the UWA Patents' claims. *See, e.g.*, Dowdy Suppl. Rebuttal ¶¶ 35-36, 44-46, 54, 61. Dr. Dowdy is incorrect. A POSA at the time of the invention would understand that these structural variabilities may affect the claimed function, and that they would need to be experimentally tested to determine their effect.

61. *Backbone Chemistry Affects Exon Skipping*: Dr. Dowdy argues, based on Gebski (2003) and Fletcher (2006) that "it was known" that converting a 2'OMe-PS ASO to a morpholino ASO targeting the same region of pre-mRNA would produce "generally translatable" exon skipping ability. Dowdy Suppl. Rebuttal ¶ 93. I disagree.

62. As for Gebski (2003), Dr. Dowdy draws a conclusion that is inconsistent with the article. The study tested only a single "morpholino AO, ψ M23D(+07-18)," and the 2'OMe-PS previously tested by the authors was "M23D(+02-18)," *i.e.*, it did not target the same region of pre-mRNA. Gebski (2003) at fig. 1, 1808. The authors explained that "as the target sites of the

two AOs differ by five nucleotides . . . generalizations regarding the superiority of the morpholino over the 2OMe PS chemistry *are purely speculative* at this stage.” *Id.* at 1808. In fact, they reportedly deemed it necessary to “expand[] this work by directly comparing a range of chemistries at the target sites to further explore the relationship of chemistry and target site sensitivity.” *Id.* at 1808-09. A POSA therefore would understand Gebski (2003) to teach that insufficient work had been conducted to generalize whether 2’OMe-PS activity was generally translatable to any other chemistry.

63. Fletcher (2006) was published after June 28, 2005, and its teachings therefore do not reflect what a POSA would have known at that time. Even if it were available, I disagree with Dr. Dowdy that a POSA would understand it to teach that 2’OMe-PS activity was “generally translatable” to morpholino ASOs. Fletcher (2006) compares *in vivo* a 2’OMe-PS and PMO each targeting M23D(+7-18)—*i.e.*, a single “target region” within exon 23. Fletcher (2006) at 208, 214. The authors concluded that “Data from RNA and immunofluorescent studies presented here show that the morpholino AO was superior to the 2OMeP AO . . . at inducing dystrophin expression.” *Id.* at 214. But they noted that low levels of skipping observed with 2’OMe-PS was “inconsistent with other reports,” and that the “ability of the uncomplexed morpholino AO to induce exon skipping was unexpectedly high *in vivo*, whereas this same preparation had previously appeared ineffective *in vitro*.” *Id.* Accordingly, a POSA would understand that although the particular result from *this testing* showed higher morpholino activity, the authors had observed the morpholino being “ineffective” in other testing. A POSA therefore would not understand Fletcher (2006) as providing conclusive proof that 2’OMe-PS activity when targeting M23D(+7-18) was necessarily translatable to a morpholino ASO, much less that 2’OMe-PS activity was generally translatable to morpholino ASOs (as Dr. Dowdy argues).

64. Further, I have reviewed Dr. Wood's reply to Dr. Dowdy relating to this issue and agree with his analysis (which I hereby incorporate by reference). *See* Wood Suppl. Reply, ¶¶ 62, 67-82. Taken as a whole, these publications show that even scientists whose skill exceeds that of the POSA could not establish generalizable trends between backbone activity by June 28, 2005. Like Dr. Wood, I find Arechavala-Gomez (2007) a telling contradiction of Dr. Dowdy's opinions. This article was co-authored by members of the research groups at UWA (including co-authors to Gebiski (2003) and Fletcher (2006)), Royal Holloway University, Leiden University (including co-authors to Aartsma-Rus (2004), and Prosensa. It acknowledged the prior Gebiski (2003) work, yet reported that "when these [post-priority-date] experiments [being reported on] were designed it was *not* clear whether one could extrapolate results between sequences when different chemistries were used." Arechavala-Gomez (2007) at 805.

65. Additionally, [REDACTED] refutes Dr. Dowdy's opinion. For example, [REDACTED]
[REDACTED]. As I explained in my opening report and shown below, [REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

SRPT-VYDS-0156085 at 6172; Adams Tr. 49:1-50:2.

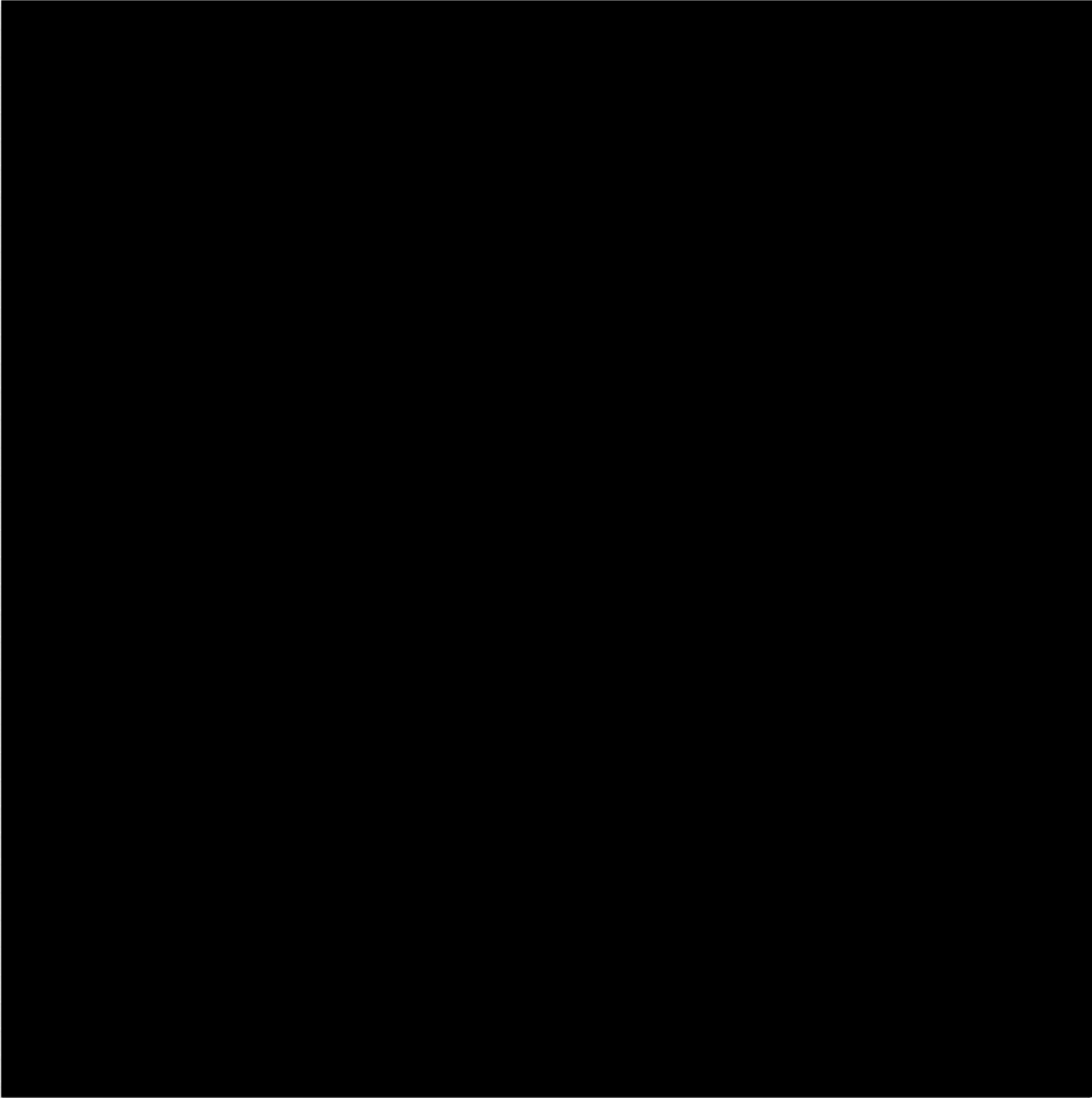
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SRPT-VYDS-0158109 at 8145 ([REDACTED]); Adams Dep. Tr. at 68:20-
69:14 ([REDACTED]);
SRPT-VYDS-0162286 at 2402 ([REDACTED]).

66. [REDACTED]:



SRPT-VYDS-0156085 at 6273; *see also id.* at 6089 (

).



SRPT-VYDS-0158291 at 8318.

67.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]. *E.g.,*

SRPT-VYDS-0162285 at 2405.

68. *Nucleobase Modifications & End Modifications*: As noted above, Dr. Dowdy implies that nucleobase modifications and end modifications would not affect exon skipping activity. I disagree.

69. Dr. Dowdy asserts, for example, that chemical moieties “are peripheral for inducing exon skipping” and only “impact properties like solubility or delivery.” Dowdy Suppl. Rebuttal ¶¶ 44, 99. I have reviewed Dr. Wood’s reply to Dr. Dowdy relating to this issue and agree with his analysis (which I hereby incorporate by reference). *See* Wood Suppl. Reply, ¶¶ 57-58. Dr. Dowdy is drawing an artificial distinction—successful delivery, cell penetration and uptake are necessary predicates to an ASO achieving exon skipping in a cell.

70. Further, I disagree with Dr. Dowdy that chemical moieties only affect solubility or delivery. The only support he cites from on or before June 28, 2005 is an out-of-context statement taken from Errington 2003, (*id.* ¶ 44 (quoting statement that ASOs are “mediated by a true antisense mechanism”), and a misreading of the specification, (*id.* ¶ 45). Regarding the former, Errington 2003’s quoted statement is a conclusion drawn from observing that “mismatched 2’OMeAO controls” had “a reduced annealing potential reflecting their weakened biological function.” Errington 2003 at 525 (nevertheless noting that there is “potential for some antisense activity” with only “partial homology to the 2’OMeAOs”). This statement is not discussing the effect of 5’ or 3’ end modifications, which Errington 2003 never mentions (much less evaluates).¹⁷

71. As for the latter, the specification explicitly states that chemical moieties may affect “the **activity** . . . of the oligonucleotide,” not just “cellular distribution” and “cellular uptake”:

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance **the activity**, cellular distribution or cellular uptake of the oligonucleotide.

’851 Patent at 27:47-51. Mechanistically, that is the case not only because chemical moieties affect ASOs’ ability to reach pre-mRNA through cell membranes (“cellular distribution” and

¹⁷ The only reference to chemical “modifications” comes in description of the 2’O-Me backbone. *Id.* at 519. Errington 2003 contains no discussion of nucleobase modifications or morpholinos either.

“cellular uptake”), but also because the additional chemical structure may interfere with their ability to anneal to the pre-mRNA. As noted in my Opening Supplemental Report (¶¶ 94-96), pre-mRNA with “extensive secondary structure” can impair an ASO’s ability to “fit” into the pre-mRNA’s three-dimensional structure so that it can anneal. This is why the UWA researchers attempted to use “computer programs . . . to identify regions of the pre-mRNA which may not have had extensive secondary structure”—they presumed such locations to be “potential sites for annealing of antisense molecules.” ’851 Patent at 32:22-36.

72. Accordingly, as of June 28, 2005, a POSA would have understood that 5’ and 3’ chemical moieties present a quintessential example of the “goldilocks” dilemma I discussed. *See* Hastings Suppl. Opening Rpt. ¶¶ 105-106. Lengthening the chemical structure of an ASO with a moiety can aid delivery of the ASO to pre-mRNA (*e.g.*, by changing the ASOs charge to allow it to better penetrate cell membranes), only for that altered chemistry to then interfere with the ASO’s ability to actually anneal to the pre-mRNA (*e.g.*, because the altered charge alters AO’s binding affinity and/or the additional chemical groups sterically impair the AO’s ability to “fit” within pre-mRNA secondary structure). Indeed, it was known before June 28, 2005 that chemical modifications along an ASO *outside* the nucleobases engaging in Watson-Crick hydrogen bonding could materially affect binding affinity. *See, e.g.*, Freier & Altmann (1997) at tbls. 3-7, figs. 1-4 (describing changes resulting from 2’ sugar modifications, 3’ substituted thymidines, 4’ oxygen substitutions, 1’ and 6’ substituted carbocyclic nucleoside analogs, and bicyclic modification or cyclobutyl substitution),

73. Dr. Dowdy further tries to argue that end modifications have no material effect by noting that different laboratories testing different chemistry for ASOs targeting exactly h53A(+23+47) each reported some degree of exon skipping. Dowdy Suppl. Rebuttal ¶ 100. Dr.

[REDACTED]

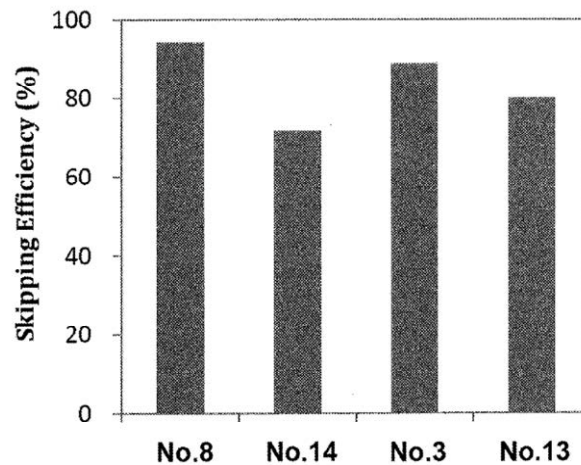
Dowdy's logic is doubly flawed. First, even if targeting h53A(+23+47) produced consistent activity across these particular chemical modifications, that does not mean it would persist across all chemical modifications. Second, that all laboratories reported *some* exon skipping does not mean that the altered chemistry did not affect exon skipping—it just means that those effects did not eradicate skipping under the particular conditions tested. Dr. Dowdy neglects to mention, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]. See *supra* ¶¶ 64-65. This clearly demonstrates that the backbone chemistry of an ASO can affect its activity.

74. The NS Patents also provide a comparative example within Dr. Dowdy's purported "hot spot." PMO Nos. 3 (targeting bases 32 to 56) and 8 (targeting bases 36 to 56) use "5' end: group (3)"—an "-OH" group. *Id.* at 25:1-30, tbl. 2. PMO Nos. 13 and 14, respectively, target the exact same sites, but use "5' end: group (1)"—a TEG group. *Id.* Within the same experiment, this additional 5' chemistry caused an observable decrease in exon skipping:



[REDACTED]

'092 Patent at Fig. 8. Because the unmodified PMO Nos. 3 and 8 were highly active, they happen to have retained exon skipping. But the same would not necessarily be true when the unmodified ASO has little to no activity itself.

75. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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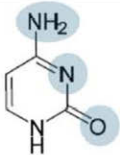

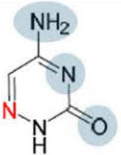
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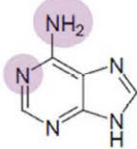
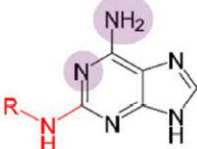

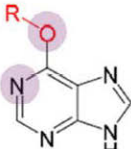
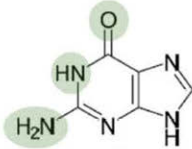
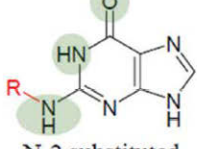
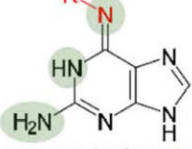
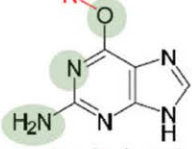
[REDACTED]

[REDACTED] These examples clearly show that modifying ASO chemistry affects exon skipping activity, and do so within Dr. Dowdy's supposed "hot spot."

76. Dr. Dowdy makes similar arguments for modified nucleobases, suggesting that because they comprise the necessary chemistry to allow a Watson-Crick base pair to occur, ASOs employing that modified nucleobase will necessarily bind pre-mRNA in precisely the same

manner as ASOs lacking that modification. Dowdy Suppl. Rebuttal ¶¶ 34-36. That argument is simply wrong. As Dr. Dowdy's annotations to my exemplary illustrations of modified bases show, nucleobase modifications literally change the chemistry around the atoms that are participating in the hydrogen bond, if not changing the atoms themselves that hydrogen bond:

Unmodified/unsubstituted	"5-substituted pyrimidines"	"6-azapyrimidines"
 cytosine	 5-substituted cytosine	 6-azacytosine

Unmodified/unsubstituted	"N-2 substituted purines"	"N-6 substituted purines"	"O-6 substituted purines"
 adenine	 N-2 substituted adenine	 N-6 substituted adenine	 O-6 substituted adenine
 guanine	 N-2 substituted guanine	 N-6 substituted guanine	 O-6 substituted guanine

"Figure 2" from Dowdy Suppl. Rebuttal, ¶ 35. A POSA as of June 28, 2005 would have understood that these chemical alterations to the bonds (*e.g.*, replacing the hydrogen-bonding atom) and/or additional steric interference (*e.g.*, when a neighboring R- group interferes with how the ASO "fits" the pre-mRNA) affect how strong the ASO's affinity for the pre-mRNA would be. *See, e.g.*, '851 Patent, at 27:37-39 (explaining that "[c]ertain nucleo-bases" "increase[e] the binding affinity of the oligomeric compounds"); Freier & Altmann (1997) at fig. 4, tbls. 2, 8-10

(describing changes resulting from 5-methyl pyrimidine substitution, 5 or 6 pyrimidine substitution, substitution of O4 or O2 of 2'-O-methyl uridine, and modified pyrimidines).

77. Dr. Dowdy cites a provisional application from Prosensa researchers that he argues “confirms that ASOs with modified bases can be used to induce exon skipping.” Dowdy Suppl. Rebuttal ¶ 98. As explained above, I do not disagree that ASOs can have modified nucleobases and still induce exon skipping. But that does not mean that, as Dr. Dowdy implies, modifying nucleobases has no bearing on exon skipping activity. In fact, the de Visser US ’354 Application he cites indicates otherwise. The modified ASOs (PS524 and PS399) produced exon skipping activity that differed from the unmodified ASOs (PS229L and PS220) by 1.9-fold and up to 10-fold, respectively. De Visser US ’534 at 63-64. Of course, this publication is both (1) post-priority date; and (2) only tests a single chemical modification (5-methylcytosines) to 2'OMe-PS ASOs, so as of June 28, 2005, it would not have provided any POSA guidance regarding how the various possible nucleobases would have affected ASOs within the claimed exon 53 genus.¹⁹

* * *

78. Because Dr. Dowdy addresses the effects of structural variations individually (*i.e.*, addressing nucleobase modifications, then 5'- and 3'- end groups), my reply follows suit as a matter of convenience. However, he appears to neglect that the claims encompass not only ASOs with only one of these structural variations, but also ASOs with ***combinations*** of them. As one example, VYONDYS 53 (golodirsen) includes both a different “target region” than any reported

¹⁹ Generalized pre-priority date work like Freier & Altmann (1997) also would have been insufficient. As the tables reflect, their work measured changes in binding affinity relative to DNA, and a POSA would understand that the corresponding change relative to morpholino backbones may differ. Further, Frier & Altmann (1997) often observed differing changes for different ASOs, demonstrating that the effects may differ from ASO to ASO, depending on its base composition.

in the UWA Patents and a 5' end modification. ASOs meeting all structural limitations could simultaneously have different lengths and nucleobase modifications too. Thus, a POSA would need to consider not only the unpredictability of a sole chemical modification, but also the added unpredictability that would come with implementing multiple changes at the same time. As of June 28, 2005, a POSA would understand that layering multiple chemical alterations onto a given ASO could either magnify the effects of individual modifications or cause them to, in effect, “cancel out,” and that testing would be required to assess the effects of such modifications together. Given that the specification discloses no such testing, a POSA would not understand it to disclose a structure-function relationship across the claimed genus.

79. Thus, it remains my opinion that the UWA Patents fail to disclose common structural features sufficient to showing possession of the claimed functional genus.

3. The UWA Patents Do Not Disclose a Representative Number of Species

80. Dr. Dowdy relies upon SEQ ID No. 195 as his sole example of a purportedly “representative” species. Dowdy Suppl. Rebuttal, ¶¶ 103-105. His argument boils down to asserting that (1) this exemplary ASO (SEQ ID No. 195) meets most claim limitations; and (2) the specification states that it could be changed to use the limitations it does not meet (*i.e.*, thymine bases and morpholino chemistry). Again, Dr. Dowdy inappropriately conflates whether SEQ ID No. 195 meet the claimed “fenceposts” with whether that sole ASO represents the full breadth of varying ASOs encompassed by the claimed genus.

81. Unlike Dr. Dowdy, I consider *both* the claimed criteria and the structural and functional variety across ASOs meeting those criteria. *See* Hastings Suppl. Opening ¶¶ 83-113. Structurally, SEQ ID No. 195 does not reflect the claimed backbone chemistry (morpholino), the claimed “thymine” bases, the permitted 5'- and 3'- end modifications, nor the permitted

[REDACTED]

nucleobase modifications. Likewise, the sequence reflects only 1 of the 12 lengths that the claimed ASOs may be and only 1 of 168 “target regions” claimed ASOs may target. Functionally, SEQ ID No. 195 does not reflect ASOs with any more than “very faint levels of skipping” (if that).²⁰ It therefore does not reflect the full range of exon skipping functionality encompassed by the claims, which includes even ASOs with far higher (even therapeutic) levels of exon skipping.

82. Dr. Dowdy’s graphic does not “appropriately account[] for” the structural and functional breadth across the claimed genus—it obscures them behind generalized claim limitations that encompass many structural and functional variations. *See* Dowdy Suppl. Rebuttal ¶ 111. A proper comparison would examine *how* the ASOs meet (or do not meet) meet the claim limitations and/or reflect other encompassed structures. That comparison shows both parties’ products to materially differ from SEQ ID No. 195:

²⁰ Dr. Dowdy’s argument that “multiple scientists testified that H53A(+23+47) successfully induced exon 53 skipping, including *after review of the underlying primary data*,” only proves my point. Dowdy Suppl. Rebuttal ¶ 108. In general, scientists afford weight to objective data proven reproducible through multiple rounds of independent testing and subsequent statistical analysis of quantified results to demonstrate significant effects that are not random. In the absence of such quantifiable data, as is the case with the UWA patent data, an independent analysis of the primary results are necessary and cannot be substituted with qualitative summaries. [REDACTED]

[REDACTED] See Hastings Suppl. Opening ¶ 138.



Encompassed Structures	SEQ ID No. 195	Viltolarsen	Golodirsen
Length	25 bases	21 bases	25 bases
Target Region	+23 to +47	+36 to +56	+36 to +60
# of “Consecutive Bases”	All 25 Consecutive	Only 12 Consecutive	Only 12 Consecutive
Backbone Chemistry	2’OMe-PS	PMO	PMO
Thymine/Uracil Bases	Uracil	Thymine	Thymine
Nucleobase Modification	None	None	None
End Modification	None	None	5’ TEG Group
Level of Skipping	“Very Faint”	Therapeutic	Therapeutic

83. Dr. Dowdy largely repeats his arguments for why failing to test SEQ ID No. 195 as a morpholino with thymine bases is supposedly immaterial, (Dowdy Suppl. Rebuttal ¶ 105), and why nucleobase modifications and 5’- and 3’- end modifications supposedly do not affect exon skipping function, (*id.* ¶ 106). I disagree for the reasons provided above.

84. Regarding the former, however, Dr. Dowdy additionally points to post-priority date testing where researchers ostensibly “confirmed” that h53A(+23+47) works in a PMO format. *Id.* ¶ 105. Dr. Dowdy’s reliance on supposedly “confirmatory” post-priority date evidence (such as this) does not support his points—it undercuts them. Because *none* of that evidence was available to a POSA as of June 28, 2005, the POSA would review and interpret the specification *without* the benefit of any guidance from post-priority date references. That Dr. Dowdy cannot point to the specification’s disclosed testing as “confirmatory” evidence for his assertions, and must instead resort to after-the-fact work conducted by others only highlights how the UWA Patents’ specification itself fails to demonstrate possession of the full scope of the claimed genus. The

collective post-priority date testing Dr. Dowdy cites does not evaluate each one of the 168 “target regions,” much less all claimed ASOs.

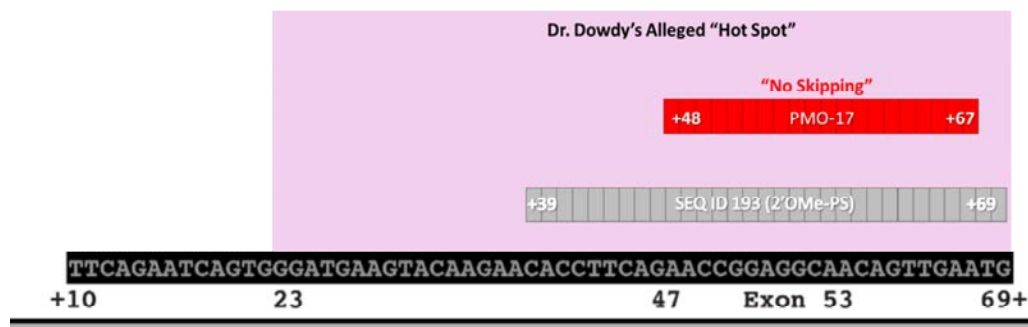
85. Thus, it remains my opinion that the UWA Patents fail to disclose representative species showing possession of the claimed functional genus.

4. Reply to Dr. Dowdy’s Other Critiques

86. [REDACTED]: I have reviewed Dr. Dowdy’s critiques (Dowdy Suppl. Rebuttal ¶¶ 114-121), but they do not change the opinions I set forth in my Supplemental Opening Report. I also wish to note my disagreement with Dr. Dowdy’s accusation that my analysis of exon 3 and 43 data is a “red herring.” As I explained in my Supplemental Opening Report, these examples refute the notion that a “hot spot” can be defined from as few as four ASOs described in the UWA Patents’ specification (as Dr. Dowdy claims).

87. CERI Experiments: I disagree with Dr. Dowdy’s analysis of the CERI experiments, including his conclusion that they “confirm that ASOs meeting the claimed structural requirements . . . predictably induce exon 53 skipping.” Dowdy Suppl. Rebuttal ¶ 126. Dr. Dowdy oversimplifies the results as if the *only* information a POSA could glean from that data is whether the ASOs did or did not induce exon 53 skipping. *See id.* ¶¶ 123, 127 (simplifying results into “yes” and “no”). That interpretation is incorrect. I designed these experiments to quantify the amount of exon skipping, which allows one to further examine whether exon skipping *varied* with changes in structural features. The results show that exon skipping *does* vary in response to changes in structural features and demonstrates that even combining claimed features (*e.g.*, being a PMO, 100% complementary, and at least 12 consecutive bases of SEQ ID No. 195) does not guarantee that an ASO will induce exon skipping.

88. Dr. Dowdy also ignores the result observed with PMO-17, which refutes the logic underlying his arguments. As discussed above, Dr. Dowdy relies upon the results for four ASOs—none of which are claimed—spanning positions +23 to +69 to define an exon 53 “hot spot” that he argues provides predictability across that entire region (such that a POSA would assume exon skipping despite the permitted variations in length, target region, etc. allowed by the claims). Of these, the UWA Patents report SEQ ID No: 193 (h53A(+39+69)) to have the most exon skipping activity. ’851 Patent, tbl. 39. PMO-17 is 100% complementary to a “target region” of consecutive bases (h53A(+48+67)) *entirely within* SEQ ID No. 193’s “target region” (and thus Dr. Dowdy’s purported “hot spot”) and yet PMO-17 exhibits “no skipping.”



If ASOs targeted not only to Dr. Dowdy’s “hot spot,” but to the portion of that “hot spot” corresponding to the *most active* exon 53 ASO disclosed in the UWA Patents exhibit no activity, a POSA would likewise conclude that ASOs targeted to portions of the “hot spot” corresponding to the *least active* exon 53 ASO disclosed in the UWA Patents (SEQ ID No. 195) would likewise exhibit no activity.

89. Additionally, as with other post-priority date evidence, Dr. Dowdy imparts too much significance from after-the-fact testing showing discrete instances of claimed ASOs exhibiting exon 53 skipping. The UWA Patents’ claims are far broader and encompass far more structural variability than the post-priority date examples he cites (including the ASOs CERI

tested).²¹ But perhaps more importantly, such data is *not* provided in the UWA Patents' specification, and does not evidence that the UWA researchers possessed the "full scope" of the claimed genus *as of June 28, 2005*. Rather, that the CERI species were identified only through experimentation conducted by another lab more than a decade later demonstrates how incomplete UWA's experimentation was at that time. To put a finer point on it, I designed experiments that quantitatively showed skipping for six claimed morpholino ASOs—the UWA researchers showed none.

B. The UWA Patents Are Invalid for Lack of Enablement

90. I have reviewed Dr. Dowdy's supplemental rebuttal opinions regarding enablement, but disagree with him. It remains my opinion that the UWA Patents are invalid for lack of enablement.

1. The Claims' Scope Is Broad

91. Regarding structural breadth, Dr. Dowdy briefly reiterates his prior arguments, which I disagree with and have replied to above. *Supra* Sections V.A.1 (addressing claim scope), V.A.2.b-c (explaining how chemical modifications demonstrably affect the claimed function).

92. Regarding functional breadth, Dr. Dowdy concedes that the claims "encompass ASOs with varying abilities to induce exon 53 skipping," but claims that this "does not change the scope of experimentation required." Dowdy Suppl. Rebuttal ¶ 134. Dr. Dowdy's opinion conflicts with the evidence of exon 53 development in this case, which demonstrates that obtaining therapeutically-effective ASOs—such as NS's accused product, VILTEPSO, and Sarepta's product, VYONDYS 53—takes additional effort beyond mere screening (as has been my

²¹ Attempting to test for all of those untested variations would have been time and cost prohibitive. *See, e.g.*, Hastings Suppl. Rebuttal, n. 38 (explaining that testing the 29 ASOs in the CERI reports took upwards of 14 months to complete).

[REDACTED]

experience). As Dr. Wilton admitted, [REDACTED]

[REDACTED] Wilton Dep. at 28:9-18 [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] To identify ASOs with therapeutically-relevant levels of exon 53 skipping in 2009-2010, Nippon Shinyaku and NCNP embarked on an iterative project involving an exon walk along the entire exon 53 followed by iterative testing of ASOs of differing lengths, target regions, and 5' caps. *See generally* Watanabe (2018); '092 Patent. Likewise, Sarepta identified VYONDYS 53 (golodirsen) after participating in a SKIP-NMD grant with various laboratories (but not UWA).²²

93. Dr. Dowdy also suggests that “a POSA would not need to test every possible candidate ASO,” and may even test fewer than the “number of target regions.” Dowdy Suppl. Rebuttal ¶ 136. Given the high level of unpredictability in the art, I disagree that such limited experimentation would enable the “full scope” of the claims, which undisputedly include ASOs with therapeutic levels of activity. As discussed above, chemical modifications affect exon-skipping activity, as do parameters such as length and target region. A POSA’s ability—if any—to extrapolate results from one ASO to another would therefore have been exceedingly limited,

²² *See Sarepta Therapeutics Announces First Patient Dosed in European Phase I/II Study of SRP-4053 in Duchenne Muscular Dystrophy Patients*, Jan. 14, 2015 (available at <https://investorrelations.sarepta.com/news-releases/news-release-details/sarepta-therapeutics-announces-first-patient-dosed-european>) (“SKIP-NMD is an EU FP7 funded collaborative grant involving 10 partners from Europe and the U.S., whose aim is to restore dystrophin production in a subset of DMD boys. This will be achieved by developing a drug which ‘skips’ the mutations causing DMD, so as to restore dystrophin protein expression.”).

and would not appreciably reduce the amount of experimentation to a level a POSA would consider “routine.”

2. The State of the Prior Art & Unpredictability

94. Dr. Dowdy argues that I “focus[] on generalized unpredictability” and not “the predictability of exon 53 skipping” in light of the specification’s disclosures. Dowdy Suppl. Rebuttal ¶¶ 139-140. I disagree. As discussed in my Opening Supplemental Report and above, there is ample evidence—including the varying activity reported in the UWA Patents’ specification—that shows that exon 53 (including Dr. Dowdy’s alleged “hot spot”) was no exception to the high levels of unpredictability in the art as of June 28, 2005. *See* Hastings Suppl. Opening Rpt. ¶¶ 87-98, 106, 108. Sarepta and UWA acknowledged that much of the evidence I rely upon is directly relevant to the claimed subject matter by relying upon it during the ’851 Patent’s prosecution (and before that, the ’007 Interference). *Id.* ¶¶ 99-108.

95. *Making Morpholino ASOs*: Dr. Dowdy disputes my opinions that making morpholino ASOs was, as of June 28, 2005, difficult, time-consuming, and expensive for a POSA, because supposedly “‘straightforward’ options were available for synthesizing PMOs, which were also available for commercial purchase.” *See* Dowdy Suppl. Rebuttal ¶¶ 141-146. Notably, however, Dr. Dowdy does not contest that a POSA would have been unable to commercially obtain 31-mer PMOs, nor that obtaining PMOs longer than 25 bases would have been difficult. *See id.* (mentioning the former argument without disputing it, and neglecting the latter); *see also* Wood Suppl. Reply ¶¶ 53-54.

96. Dr. Dowdy argues that “a POSA would have been able to purchase the vast majority of claimed PMOs.” *Id.* ¶ 181. Even setting aside the difficulties with unmodified morpholino ASOs, this opinion ignores the undisputed fact that claimed ASOs may include 5’ or 3’ end

modifications and/or nucleobase modifications. Dr. Wood and Dr. Dowdy agree that, as of June 28, 2005, Gene Tools offered no more than five 3'-end modifications. *See* Wood Suppl. Rpt. ¶ 29 (citing contemporaneous images of Gene Tools's website); Dowdy Suppl. Rebuttal ¶ 181, n. 13; *see also* Wood Suppl. Reply ¶ 55. Dr. Dowdy does not contest that morpholinos with 5' end modifications were not available through Gene Tools as of June 28, 2005. *Compare* Wood Suppl. Rpt. ¶ 29 (asserting same); Wood Suppl. Reply ¶ 56, *with* Dowdy Suppl. Rebuttal ¶ 181, n. 13 (offering no rebuttal). And neither the Gene Tools website nor Dr. Dowdy provide any indication that morpholinos with modified nucleobases were likewise available. *See* Wood Suppl. Rpt. ¶ 29 (citing contemporaneous images of Gene Tools' website); Dowdy Suppl. Rebuttal ¶ 181, n. 13; *see also* Wood Suppl. Reply ¶ 59. Thus, a POSA would understand that, as of June 28, 2005, the vast majority of morpholino ASOs that meet all structural requirements of the UWA Patents' claims would **not** have been commercially obtainable.

97. Regarding making morpholinos, Dr. Dowdy's only support for his argument that making morpholinos (or morpholino "monomers") would have been "straightforward" to a POSA are cherry-picked statements from publications by the co-inventor of morpholinos, James Summerton. *See id.* ¶¶ 143-144 (citing Summerton (1997), Summerton (2003), and Summerton US '444). These do not prove his point. Dr. Summerton and his company (Gene Tools) were perhaps the world's foremost experts on morpholino synthesis at the time. What they considered "relatively straightforward" is not reflective of how one of **ordinary** skill viewed morpholino synthesis at the time.

98. Absent from Dr. Dowdy's report is any evidence that chemically synthesizing morpholino ASOs was, as of June 28, 2005, within the level of **ordinary** skill. As Dr. Wood points out, all of Dr. Dowdy's cited literature on the use of morpholino ASOs "reflect[s] that scientists

working with PMOs before and during the 2005 time frame were either affiliated with Gene Tools or AVI BioPharma (e.g., James Summerton, Pat Iversen), or obtained their PMOs from one of those companies.” Wood Suppl. Reply ¶¶ 43-45, 47; *see also id.* ¶ 46 (noting that contemporaneous pharmaceutical companies Prosensa and Isis (now Ionis) forced on non-morpholino chemistries).

99. As Dr. Wood also points out, making morpholino ASOs with chemical modifications would have particularly difficult and “resource-intensive.” Wood Suppl. Reply ¶¶ 55-56. It is telling that even Gene Tools, on its public website, explained that it “cannot modify the 5’ end of a Morpholino oligo” “due to synthesis constraints.”²³ Such a statement from the inventors of the morpholino chemistry highlights that making the full range of structurally-distinct morpholino ASOs within the claimed genus would have been difficult for even those with skill far exceeding ordinary levels.

100. I also disagree with Dr. Dowdy that a POSA could simply “partner with other laboratories or set up the requisite synthetic processes to make morpholinos.” Dowdy Suppl. Rebuttal ¶ 145. Dr. Dowdy attempts to justify his speculation by citing testimony from Dr. Wilton. *Id.* (citing Wilton Dep. at 34:15-36:12). But Dr. Wilton’s experience comports with my and Dr. Wood’s opinions that morpholinos were expensive and difficult to obtain. According to Dr. Wilton,

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

²³ <https://web.archive.org/web/20051222184949/http://www.gene-tools.com/node/23>.

██████████ *Id.* at 31:18-32:10. This demonstrates that making and obtaining morpholino ASOs was a material impediment to those of skill in the art—even those in prominent laboratories (like Dr. Wilton) for whom a partnership with a company like AVI may have been feasible. Further, AVI itself had limited resources. For example, in 2011, ██████████
██████████. WILTON0018486 at 489.

101. Testing Morpholino ASOs: Dr. Dowdy agrees that, as of June 28, 2005, successfully “deliver[ing] PMOs to cultured cells was a well-known problem,” (Dowdy Suppl. Rebuttal ¶ 147), and he does not contest that “consistent techniques to assess dystrophin exon skipping both *in vitro* and *in vivo* had not yet been established,” (*id.* ¶ 149). Instead, Dr. Dowdy notes that the POSA is defined as being generally familiar with “delivery methods for antisense oligonucleotides” and “cell-free, cell-based and/or *in vivo* experiments, as well as DMD models,” and argues that a POSA “would have known how to account for” these problems. *See id.* ¶¶ 148, 150.

102. Dr. Dowdy’s critique misses the point. That neither the art nor the specification provided POSAs with a reliable, well-established starting point for their experimentation (*i.e.*, that POSAs would instead encounter and need to “account for” these transfection and assay problems before they could reliably evaluate ASO candidates) demonstrates that making and using the full scope of the claimed inventions would not have been a simple or routine task. Put differently, when Dr. Dowdy argues that a POSA would have “account[ed] for any potential variability stemming from different transfection conditions by, for example, designing adequate leashes or using Endo-Porter,” and “account for assay variability by choosing an appropriate assay and experimental condition,” (*id.* ¶¶ 148, 150), what he is doing is describing the types of *additional* experimentation that the nascent state of the art would have necessitated for a POSA attempting to make and use the full scope of the claimed genus.

103. I also disagree with Dr. Dowdy's characterization of POSAs' attempts to solve these problems before June 28, 2005 as purportedly providing "solutions." As Dr. Wood correctly points out, the prior art approaches "varied in feasibility and effectiveness," and merely represented efforts to "explor[e]" how to "assess[] morpholino AONS for exon skipping in cells *in vitro* and systematically *in vivo*." Wood Suppl. Rpt. ¶¶ 38, 43; Wood Suppl. Reply ¶¶ 61-64 (explaining the inapplicability of the existing leashes to exon 53 and unsuitability of *in vitro* methods to *in vivo* work). The struggles experienced by the UWA researchers even after June 28, 2005 is particularly notable, as it demonstrates that the named inventors were no exception. *See id.* ¶ 46 (explaining how the UWA researchers reported experiencing issues with poor cellular uptake that affected their ability to evaluate PMOs for exon skipping activity). As Dr. Wood points out, the Aatsma-Rus (2004) authors from Leiden University also encountered difficulties despite their relatively higher levels of skill. Wood Suppl. Reply ¶ 62.

104. Dr. Wood also correctly points out that "[t]he papers Dr. Dowdy cites as providing 'detailed protocols for conducting exon skipping assays' were not for PMOs." Wood Suppl. Reply ¶ 65 (explaining how he reported on the lack of agreement on "parameters for *in vitro* AO screening" even years later, in a 2010 article).

105. Experimental Variability v. Unpredictability: Dr. Dowdy incorrectly implies that Dr. Wood and I are relying upon minor experimental variabilities one might observe assay to assay, and leverages that to accuse us of conflating "unpredictability" with "experimental variability." He is incorrect. Science fundamentally relies upon using reliable methods to obtain replicable results in order to disprove the null hypothesis (that hypothesizes there is no relationship between variables; *i.e.*, no activity in this case).

106. When a field is in its infancy, and scientists are still attempting to establish reliable methods for testing (as was the case for exon 53 skipping ASOs as of June 28, 2005), it limits their ability to obtain replicable results. Inconsistent—*i.e.*, non-replicable—results are not unusual, and yet a POSA has fewer tools at their disposal to resolve those inconsistencies. This replicability issue calls into question the veracity of results a POSA may have obtained, and thereby erodes their predictive value. *See also* Wood Suppl. Reply ¶ 28 (“When experimental variability is high, a POSA would not be able conclude whether either a negative or a positive exon skipping result was a true result if it is not reproducible.”). As Dr. Wood succinctly puts it, “[e]xperimental variability is distinct from unpredictability,” but inconsistencies across experiments or assays “compound[] unpredictability.” Wood Suppl. Reply ¶¶ 24-25.

107. *Gebski (2003) and Aartsma-Rus (2004)*: I have replied to Dr. Dowdy’s critique regarding these references above. *See supra* Section V.A.2.c.

3. The Specification Provides Little to No Guidance and Working Examples

108. Dr. Dowdy largely reiterates his critiques regarding the alleged transferability of 2’O-Methyl activity to morpholino ASOs as of June 28, 2005. I have replied to Dr. Dowdy’s critique regarding these issues above. *See, e.g., supra* Section V.A.2.c., V.A.3.

109. Dr. Dowdy argues that the transfection reagent I used in the CERI studies was available as of June 28, 2005. Dowdy Suppl. Rebuttal ¶ 159. I believe he is mistaken. I did not use “Endo Porter,” I used a different formulation called “Endo Porter DMSO.” *See, e.g.*, NS00102988 at 002 (Study Number: 936-21-M-0644) (“Sixty µL of *Endo-Porter DMSO* was added . . .”); NS00103061 at 076 (Study Number: 936-22-M-0661) (same). To my understanding,

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this formulation did not appear on Gene Tools’s price list until the April 10, 2006 version issued.²⁴ The August 2, 2004 Gene Tools price list Dr. Dowdy cites references “Endo-Porter (for 50 mL of medium),” whereas the April 10, 2006 price list now states that “DMSO and aqueous version available.”²⁵

110. Additionally, I disagree with Dr. Dowdy that “other effective PMO transfection methods were known before June 2005.” Dowdy Suppl. Rebuttal ¶ 159. Gebski’s (2003) leashes were designed for ASO sequences outside of exon 53, and would not work for exon 53 ASOs—a POSA would need to design entirely new exon 53 leashes if they were to use that general approach. Further, as discussed above, *supra* Section V.B.2, I disagree that POSAs’ attempts to solve the PMO uptake problems before June 28, 2005 providing “effective . . . methods.”

4. A Vast, Undue Amount of Experimentation Would Be Required to Make and Use the “Full Scope” of the Claimed ASOs

111. Dr. Dowdy does not actually evaluate how much time it would take for a POSA to “make and use” the *full scope* of the claimed inventions. Rather, Dr. Dowdy only opines on the time it would take a POSA to employ either (1) “a rational design strategy”; or (2) “making and testing one PMO per target region,” and then “make and test additional PMOs . . . for specific purposes.” Dowdy Suppl. Rebuttal ¶ 161. This ignores the true breadth of the claimed genus, which encompasses no fewer than tens of thousands of chemically distinct antisense oligonucleotides, and likely at least millions. *See supra* Section V.A.1; Hastings Suppl. Opening

²⁴ See <https://web.archive.org/web/20060619224633/http://www.gene-tools.com:80/node/15> (Wayback Machine capture of website from June 19, 2006).

²⁵ Even if Dr. Dowdy were correct that the particular Endo Porter formulation I used was commercially available as of June 28, 2005, it would not change my opinions. As Dr. Wood points out, the article reporting Endo-Porter to be safe and effective *in vitro* was not published until November 2005, such that it would not be considered an established method to POSAs as of June 28, 2005. *See* Wood Suppl. Reply ¶ 63, n. 22.

Rpt. ¶¶ 74-81. It is also scientifically flawed. As discussed throughout my reports, given the high levels of unpredictability in the field, limited guidance in the art, and the material effects that structural changes have on the claimed function (exon 53 skipping), I disagree that a POSA as of June 28, 2005 could have reliably extrapolated results from a small number of tested ASOs (and only one pseudo-species) to other untested, chemically distinct ASOs.

112. I find it notable that, even after short-cutting the POSA's task to making and using far less than the "full scope" of the claimed genus, Dr. Dowdy opines that it would still take a POSA at least "*several months*" to conduct that experimentation. Dowdy Suppl. Rebuttal ¶ 161. Several months of unprecedented, iterative experimentation is *not* routine or a "short project."²⁶ Dr. Dowdy tellingly offers no rebuttal to paragraph 216 of my Supplemental Opening Report, in which I opine that "obtain[ing] data from testing a few dozen exon 53 PMOs" would have been "then-unprecedented work . . . worthy of publication in major journals" or sufficient to "justify awarding a graduate student their Ph.D."

113. Further, Dr. Dowdy's estimate of "several months" appears to artificially low due to incorrect math. *See id.* ¶ 166 (claiming that it "would take at most 42 weeks"). If one assumes (as Dr. Dowdy does) that 12 PMOs are simultaneously synthesized and tested per experiment, and that experiments are conducted iteratively until the 168 target regions are each tested, it would

²⁶ Dr. Dowdy does not factually dispute that, as of June 2005, testing for only "two morpholino AONs for dystrophin exon skipping activity" had been reported and that "[t]esting even ten morpholino AONs for exon skipping would have been, at the time, completely unprecedented." *See id.* ¶ 207 (acknowledging assertions without offering any contrary evidence). He likewise does not factually dispute that, "even years after the filing date, researchers continued to test only a handful of morpholino AONs at a time." *Id.* ¶ 208 (same). He also does not factually dispute that the most exon 53 ASOs any POSA had, at the time of the invention, ever reported testing in a single set of experiments was twelve, or that testing *any* number of morpholino ASOs targeted to exon 53 would have been, as of June 28, 2005, entirely unprecedented. Hastings Suppl. Opening ¶¶, 68-69, 214.

require fourteen separate experiments—not “seven” ($168 / 12 = 14$)—to test all the “target regions.”²⁷ *See id.* Applying 4-6 weeks for each “experiment” (as Dr. Dowdy did), the time to test just the unmodified “target regions” would range from **56 weeks** ($14 \times 4 = 56$) to **84 weeks** ($14 \times 6 = 84$). Thus, even ignoring the full breadth of ASOs encompassed by the claims and focusing on just the “target regions,” Dr. Dowdy is contemplating experimentation that would take between roughly 1-2 years.

114. I also disagree with Dr. Dowdy’s suggestion that Li (2021) somehow supports his opinions. Dowdy Suppl. Rebuttal ¶¶ 162-163. As Dr. Dowdy points out, (*id.* ¶ 162), Li (2021) notes that scientists—as of **2021**—perform PMO synthesis with “automated systems.” But Dr Dowdy omits the following sentence, which explains that the theoretical benefits of such “automated systems” still had **not**—as of 2021—succeeded in “improving access to PMO compounds”:

In theory, such instruments that combine automation with flow chemistry can mix and heat reagents with efficiencies that are unattainable by batch methods, but application of these potential advantages to PMO synthesis has remain[ed] elusive. Further work is needed to improve access to PMO compounds.

Li (2021) at 2-3.

115. Dr. Dowdy also seems to imply that the PMO synthesizer machines discussed in Li (2021) would have been available to a POSA as of June 28, 2005. *See* Dowdy Suppl. Rebuttal ¶ 163 (critiquing my contrast of synthesis times using Li (2021)’s “improved methodology” to the time of the invention by asserting that POSAs “could make multiple PMOs simultaneously using multiple synthesizers”). Dr. Dowdy, however, cites **no example** of a morpholino synthesizer

²⁷ I suspect that Dr. Dowdy took the number seven from paragraph 215 of my Supplemental Opening Report, where I compare the 168 “target regions” of the ’851 Patent to the twenty-four PMOs for exon 53 that Popplewell reported testing years after the priority date.

machine that was ostensibly available as of June 28, 2005, much less any examples of a POSA using such a machine.²⁸ He therefore has no basis to opine that POSAs could have obtained any morpholino synthesizer, much less multiple.

116. Indeed, the scientific literature confirms that automated PMO synthesis on machines is a relatively recent development from more than a decade after June 28, 2005. *See, e.g.,* Kundu (2022) at Abstract (reporting “for the first time the adaptation of regular PMOs in a commercial DNA synthesizer”), 9473 (explaining that “PMO synthesis in automated DNA synthesizers is underexplored,” “automated synthesis was not a norm for them” and that “PMOs are only available from the original inventors”), 9475 (“To the best of our knowledge, this is the first report to synthesize PMO sequences having more than 20 bases by an automated oligo synthesizer.”) (“In conclusion, to the best of our knowledge, we are . . . the third report by using an automated synthesizer” after Caruthers (2019), Caruthers (2020) and Li (2021)). As noted above, the contemporaneous literature instead “reflect[s] that scientists working with PMOs before and during the 2005 time frame were either affiliated with Gene Tools or AVI BioPharma (e.g., James Summerton, Pat Iversen), or obtained their PMOs from one of those companies.” Wood Suppl. Reply ¶¶ 43-45, 47.

117. Dr. Dowdy’s arguments regarding modified bases and end modifications is similarly flawed. Dowdy Suppl. Rebuttal ¶ 164. Again, he points only to Dr. Summerton’s / Gene Tools’s work, while ignoring that scant few of the chemical modifications encompassed by the

²⁸ To support the proposition that it is “common to run synthesis reactions on automated systems,” Li (2021) cites U.S. Patent No. 8,299,206, which is an AVI Biopharma patent to Dr. Weller filed years after June 25, 2005. As noted above, this simply illustrates the continuing Gene Tools / AVI Biopharma expertise with making morpholino ASOs after the priority date—it does not reflect the capabilities of one of ordinary skill.

UWA Patents’ claims would have been practically obtainable to any POSA (either commercially or through their own synthesis). *See supra* Section V.B.2.

118. I have addressed Dr. Dowdy’s incorrect view that the 168 “target regions” are somehow comparable in scope to the work conducted in the years before and after June 28, 2005 above. *See supra* Section V.A.1.d.

119. For these reasons, it remains my opinion that making and using the full scope of the claims would require undue experimentation, and that the UWA Patents are invalid for lack of enablement.

VI. CONCLUSION

120. For the reasons stated herein (and my initial Reply Report incorporated by reference), it remains my opinion that the UWA Patents are invalid for lack of written description and enablement.

Exhibit 18

**IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE**

**NIPPON SHINYAKU CO., LTD.,
Plaintiff,**

v.

**SAREPTA THERAPEUTICS, INC.,
Defendant.**

C.A. No. 21-1015 (GBW)

**SAREPTA THERAPEUTICS, INC. and
THE UNIVERSITY OF WESTERN
AUSTRALIA, Defendant and Counter-
Plaintiff**

v.

**NIPPON SHINYAKU CO., LTD. and
NS PHARMA, INC., Plaintiff and
Counter-Defendants.**

EXPERT REPLY REPORT OF DR. MATTHEW J.A. WOOD

October 27, 2023



Matthew J.A. Wood, F. Med. Sci., MA, D.Phil

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I. INTRODUCTION AND ASSIGNMENT

1. I was retained by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) in the above-captioned case as an independent technical expert.

2. In connection with my engagement, I have issued two reports: an opening report on September 8, 2023 in which I provided opinions concerning the state of the art of exon skipping therapies for treatment of Duchenne muscular dystrophy (“DMD”) and what a person of ordinary skill in the art would have understood the inventors of the asserted patents to have invented based on the Specification (the “Opening Report”).

3. On October 11, 2023, I issued a rebuttal report (“Rebuttal Report”) responding to the opening expert report of Dr. Steve F. Dowdy, Ph.D. (“Dowdy Opening”). I have reviewed certain portions of the October 11, 2023 Rebuttal Expert Report of Steven F. Dowdy, Ph.D. (“Dowdy Rebuttal”), and submit this reply report to Dr. Dowdy’s opinions. I understand from counsel for NS that I may be provided additional information as this case proceeds. Accordingly, I may need to change or augment my analysis and opinions in light of any new information or evidence that is presented after this Reply Report. I expressly reserve the right to do so.

4. I hereby incorporate my Opening Report and Rebuttal Report by reference herein.

5. In considering and forming my opinion, I have reviewed and analyzed the information and materials identified in this Reply Report. As stated, a list of the materials I reviewed in preparation of this Reply Report is attached as Exhibit 1. I have also used the applicable legal principles that were explained to me and are set forth in my Opening Report and Rebuttal Report, including applying the parties’ similar definitions of a person of ordinary skill in the art (“POSA”) in the relevant time frame.

II. REPLY TO DR. DOWDY

A. The Field of Exon Skipping Was Unpredictable in 2005 and Remains Unpredictable.

6. As set forth collectively in my Opening and Rebuttal Reports (together, my “Reports”), a POSA reviewing U.S. Patent Nos. 9,994,851 (“the ’851 Patent”), 10,227,590 (“the ’590 Patent”), 10,266,827 (“the ’827 Patent”) (collectively, the “UWA Patents”), or PCT/AU2005/000943 published as WO2006/000057 (“Wilton PCT ’057”), which share a substantively identical specification (the “UWA Specification”), would not have understood that the inventors had recognized or appreciated the invention set forth in the claims of the UWA Patents as of the June 28, 2005 priority date.¹ My opinion is informed by the consensus in the field that designing AONs for exon skipping was highly unpredictable and remains so to this day.

7. Dr. Dowdy claims that it is unclear what Section IV of my Opening Report is intended to address. Dowdy Rebuttal ¶¶ 456, 457. That should be apparent from the title of that section: “Background Regarding the Scope and Content of the Prior Art at the Time of the Claimed Invention.” Dr. Dowdy claims that citations to articles published after June 28, 2005 rendered my intention for this section unclear. Dowdy Rebuttal ¶ 457. I disagree.

8. The post-priority date articles referenced in this section include my own review article, which provides a historical overview and background information on splicing therapy for neuromuscular disease, and research reports that further support my opinions that a POSA as of

¹ Dr. Dowdy accuses me of speculating as to the inventors’ state of mind. Dowdy Rebuttal ¶ 454. I have not. As is plain from my Reports, I have provided my objective opinions from the perspective of a POSA in the relevant timeframe, i.e., 2005 for the UWA Patents and 2011 for claims 1-3 of U.S. Patent No. 10,385,092 (“the ’092 patent”); claims 1-2 of U.S. Patent No. 10,407,461 (“the ’461 patent”); claims 1-2 of U.S. Patent No. 10,487,106 (“the ’106 patent”); claims 1-12 of U.S. Patent No. 10,647,741 (“the ’741 patent”); claims 1-4 of U.S. Patent No. 10,662,217 (“the ’217 patent”); and claims 1-4 and 6-9 of U.S. Patent No. 10,683,322 (“the ’322 patent”) (collectively, the “NS patents”).

June 28, 2005 would have been familiar with the lack of predictability and reproducibility of exon skipping assays. That this unpredictability persisted after 2005—as evidenced by post-priority date publications discussed in my Reports—illustrates the state of the art as of the priority date of the UWA Patents and refutes Dr. Dowdy’s claim that the UWA Patents alleviated this unpredictability.

9. I note that during prosecution of the application issuing as the ’851 Patent, and other applications claiming priority to Wilton PCT ’057, Sarepta/UWA made similar arguments in support of the patentability of their claims over the prior art. For example, in a January 5, 2018 Amendment stating “at the time the instant invention was made, there was a significant level of unpredictability associated with selecting specific antisense oligonucleotide sequences to induce effective dystrophin exon skipping” and “the recognition of the lack of predictability in the field of exon skipping continued beyond 2005” going on to discuss the same three papers that I discuss in paragraphs 78-79 and 81 of my Opening Report, namely Arechavala-Gomez et al. *Hum. Gene Ther.*, 18(9): 798-810 (2007), Aartsma-Rus et al., *Mol. Ther.*, 17(3):548-553 (2009), and Wu et al., *PLoS One* 2011; 6(5);e19906. *See* SRPT-VYDS-0002984 at 4790, 93-95. Sarepta and UWA stated “[i]n summary, the ... Aartsma-Rus and Wu *et al.* references, along with the Decision on Motions in the ’007 interference, serve to illustrate the unpredictability associated with selecting *specific* antisense oligonucleotides that are effective for inducing skipping of dystrophin exons.” SRPT-VYDS-0002984 at 4797 (emphasis original).

10. These three references were also discussed in a February 6, 2015 Amendment in Response to Non-Final Office Action in U.S. application no. 14/317,952, an application with

claims directed to exon 53 skipping AONs.² In that response, after discussing these references, Sarepta/UWA stated “[t]he art shows that studies performed before, and long after, the date of Applicants invention demonstrated that small changes in nucleotide sequence in overlapping antisense oligonucleotides have unpredictable effects. Thus, there was a significant level of unpredictability associated with selecting a specific antisense oligonucleotide to induce effective exon skipping of human dystrophin pre-mRNA.” 2015-02-06 Amendment at p. 14.

11. I understand that Dr. Dowdy has characterized my assessment of the unpredictable nature of the field as based on AONs directed to exons other than exon 53. My Rebuttal Report makes clear that exon 53 is no exception to the general rule, even after the publication of the UWA Specification as Wilton PCT ’057. Further, as the February 6, 2015 Amendment illustrates, Sarepta/UWA also did not believe the selection of AONs to induce skipping of exon 53 had become less unpredictable after the publication of the UWA Specification.

B. The UWA Specification Did Not Resolve the Unpredictability in the Field

12. Dr. Dowdy states that “[t]he *claims* of the Wilton Patents identify multiple structural features that collectively confer the claimed function of inducing exon 53 skipping: (1) ‘antisense oligonucleotides’; (2) ‘20 to 31 bases’; (3) ‘comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA’; (4) ‘the base sequence comprises at least 12 consecutive bases of ... (SEQ ID NO:195)’; (5) ‘in which uracil bases are thymine bases’; and (6) ‘wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide.’” Dowdy Rebuttal ¶ 473. What Dr. Dowdy does not

² This application issued as U.S. Patent No. 9,035,040 with claims directed to “[a]n antisense oligonucleotide of 25 nucleotides comprising at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which cytosine bases are 5-methylcytosine bases, wherein the antisense oligonucleotide is a 2'-O-methyl phosphorothioate oligoribonucleotide, and wherein the antisense oligonucleotide specifically hybridizes to an exon 53 target region of the human dystrophin pre-mRNA to induce exon 53 skipping.”

acknowledge is that the *claims* of the UWA Patents identifying these features in the context of exon 53 skipping were not filed until September 2017 (at the earliest) and therefore are not part of the disclosure of the UWA Patents as of June 2005.

13. Notably, the UWA Specification nowhere states that the inventors had discovered or identified a “hot spot” or amenable region for exon 53 skipping. The phrases “amenable region” and “hot spot” are not used in the UWA Specification. Nor does the UWA Specification include sufficient disclosure such that a POSA would understand the inventors possessed, recognized, or appreciated that there was such “hot spot” or amenable region spanning +23 to +69 downstream of the acceptor site or that 20 to 31 bases or that at least 12 consecutive bases of SEQ ID NO: 195 are among six structural features that collectively confer exon 53 skipping activity on antisense oligonucleotides (“AONs”). *Cf.* Dowdy Rebuttal ¶ 473. These purported structural characteristics were not identified until the application issuing as the ’851 Patent was filed in September 2017.

14. A POSA reviewing the UWA Specification upon its publication as Wilton PCT ’057 would not have understood the inventors to have discovered, possessed, recognized, or appreciated any general properties of exon 53 skipping AONs that alleviated or resolved the unpredictable nature of designing such AONs to induce exon 53 skipping, much less the structural characteristics recited in the claims filed in the 2017 application that issued as the ’851 Patent.

1. The UWA Specification Does Not Disclose a “Hot Spot” or Amenable Region Spanning +23 to +69 of Exon 53 to a POSA

15. Dr. Dowdy concedes (as he must) that the UWA Specification does not use the terms “hot spot” or “amenable region,” and instead attempts to conflate the UWA Specification’s general discussion of the need to have a “selected target” or target region—*i.e.*, a segment of the pre-mRNA to which an AON is complementary and to which it may be capable of binding. Dowdy Rebuttal ¶¶ 467, 471. References to the *need* for a “selected target” (which is true for every AON),

is very different from discussing or purporting to identify a “hot spot” or “a discrete region within exon 53 that is amenable for exon skipping” which Dr. Dowdy states is “sometimes referred to as the ‘hot spot.’” Dowdy Report ¶ 99. The meaning of “hot spot” as Dr. Dowdy uses it is different from a “target region” which I understand the Court has construed to have its “plain and ordinary meaning, which means ‘a segment of the pre-mRNA.’” Opening Report ¶ 126. The Court’s construction is consistent with how Sarepta/UWA used the phrase and not how Dr. Dowdy uses it to imply an expectation of skipping activity. *Compare* March 21, 2014 Response submitted in U.S. application no. 13/902,376, at p. 33 (“As shown in Table 39, Applicants describe 3 target sequences on exon 53, H53A(+39+62), H53A(+39+69), and H53A(+45+69), which are complementary to the base sequence ... (SEQ ID NO: 192) Each of these *target regions* includes a region of 18 bases having 100% complementarity to the sequence ... (SEQ ID NO: 192).”) (emphasis added) *with* Dowdy Rebuttal ¶¶ 467,471.

16. Dr. Dowdy claims that the “relative strength of skipping ... is immaterial to the identification of the hotspot.” Dowdy Rebuttal ¶ 470. This claim is inconsistent with how Dr. Dowdy used the phrase “hot spot” to reference a region where a POSA could predict exon skipping activity, where the usual unpredictability of exon skipping is suspended, and where other researchers would accordingly focus their work. *See, e.g.*, Dowdy ¶¶ 99, 105, 116, 417; Dowdy Rebuttal ¶ 472. To identify a “hot spot” within exon 53—assuming one exists—would either require evidence of very many effective AONs of overlapping or non-overlapping sequence, varying length and varying chemistry, or elucidation of the underlying structural and/or mechanistic features, or both. Under Dr. Dowdy’s newly flexible characterization of a “hot spot,” the target region of any exon 53 AON that induced any level of exon skipping in any assay under any conditions could be considered a “hot spot.” This is misleading because as used by Dr. Dowdy,

“hot spot” implies a degree of activity and certainty for AONs complementary to the “hot spot.”

17. Even with Dr. Dowdy’s flexible and expansive definition, as discussed in my Rebuttal Report, a POSA would not have viewed the UWA Specification as disclosing or identifying a “hot spot” or amenable region for exon 53 skipping. Rebuttal Report ¶¶ 19-30. For the same reasons, a POSA would not have understood the inventors to have invented³ such a “hot spot” or amenable region. Further, the UWA Specification does not disclose sufficient information for a POSA to conclude that the boundaries of this “hot spot” or amenable region to be +23 to +69 bases from the exon 53 acceptor splice site, or that the inventors had invented these boundaries.

18. Dr. Dowdy mischaracterizes my description of the information provided in the UWA Specification as to the testing methodology used by the inventors as “detailed.” Dowdy Rebuttal ¶ 459. As set forth in my Reports, the disclosure in the specification is lacking in experimental details. Opening Report ¶ 129; Rebuttal Report ¶ 23. These details, including the incubation times and transfection reagents, controls and concentrations tested, and the number of replicates, would have been important to a POSA’s evaluation of the information reported in the UWA Specification, and determination of what conclusions should or could be drawn. *See* Rebuttal Report ¶ 23. For example, data presented in peer-reviewed publications at the time were typically drawn from experiments that were repeated multiple times in order to show they were representative and reproducible. This was often expressly stated in the materials and methods section, along with additional details that are lacking from the UWA Specification. *See, e.g.,* Harding et al., *Mol. Ther.* 15(1):157-66 (2007) (“Harding 2007”) at 165. The absence of such details are not “nitpicks”; they represent the process by which reliable scientific evidence is

³ When I use the term “invented” I am referring to what a POSA would have understood the inventors possessed, recognized, appreciated, conceived, or had a definite and permanent idea of as of the June 28, 2005 filing date of the UWA Patents.

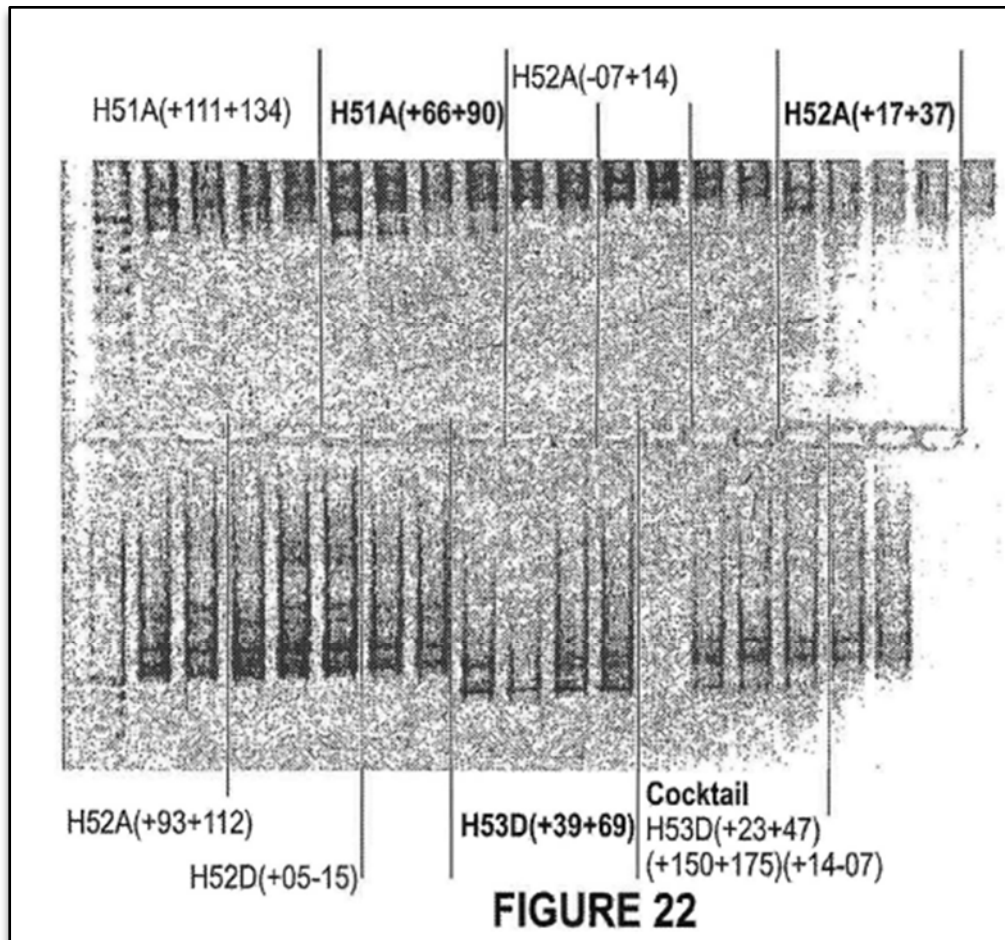
gathered and knowledge advanced and are directly relevant to the weight and conclusions a POSA would draw from the UWA Specification. *Cf.* Dowdy Rebuttal ¶ 460.

19. Further, the inconsistencies between the UWA Specification's written description of the concentrations purportedly tested and Figure 22 would have affected what a POSA understood the inventors possessed, recognized or appreciated as of June 28, 2005. The UWA Specification states that exon 51 AONs were tested at 25, 50, 100, 300 and 600 nM, exon 52 AONs were tested at 50, 100, 300 and 600 nM, and exon 53 AONs were tested at 5, 50, 100, 300 and 600 nM. '851 Patent at 62:39-44, 63:39-49, 64:38-50. A POSA would take the UWA Specification at face value and understand these concentrations were in fact tested and used to generate Table 39.⁴

20. However, a POSA would not have been able to infer what control(s) were used by the inventors in their exon 53 experiments based on other figures in the UWA Specification. *Cf.* Dowdy Rebuttal ¶ 460. Unlike Figure 22, which includes the only exon 53 skipping data a POSA could independently evaluate, other figures in the UWA Specification have lane labels. However, the inventors did not always include a control lane. *See, e.g.,* Fig. 4, Fig. 8A. Other figures have lanes labeled "UT," "neg cont." and "L2K" – sometimes on the same gel. Fig. 9A, 9B, 13, 16. Thus, I disagree with Dr. Dowdy that a POSA would have (or could have) reasonably inferred "that testing generally included an untreated negative control," much less what control was used for the exon 53 experiments, and which lanes in Figure 22, if any, are control lanes. Dowdy Rebuttal ¶ 460.

⁴ If the inventors had not in fact tested the exon 53 AONs at each of the concentrations stated in the UWA Specification, this would certainly affect a POSA's understanding of what the inventors had invented, as well as the weight (or lack thereof) he or she would assign to the scant results reported in Table 39 because those results are impossible to independently evaluate. Anything casting doubt on the reliability or accuracy of the inventors' subjective reporting would be material to a POSA's consideration of the UWA Specification.

21. Figure 22 has other issues beyond the lack of lane labels and apparent lack of control lane(s). These issues would affect how much a POSA would rely on the results in Figure 22 and the UWA Specification overall. For example, as shown below, some lanes are heavily smeared, one of the H53D [sic] (+39+69) lanes is missing the unskipped transcript, and some lanes are missing bands altogether. These technical issues with the gel would suggest to a POSA that the reported data may not be altogether reliable.



22. Even if accepted at face value by a POSA, Figure 22 is difficult to interpret. Figure 22 includes lanes for exon 51, 52 and 53 (all unlabeled). Absent lane labels, a POSA might assume the lanes are in order by concentration tested, either ascending or descending. However, this assumption is undercut by the gel itself. For example, the unskipped band(s) for H53D

[sic](+39+69) in lane 2 are missing. Either they are genuinely missing, in which case exon skipping is 100% or their absence reflects technical issues. Complete exon skipping is highly unlikely and virtually unheard of, therefore the latter is the more likely explanation. Moreover, because lane 2 would not be the highest concentration if the lanes were loaded in order by concentration tested, a POSA would expect to see this level of exon skipping in the lane that does reflect the highest concentration, lanes 1 or 4, but instead the bands in these lanes look very similar to each other (as well as lane 3), despite the presumably 120-fold difference in AON concentration tested. This is in turn problematic because a POSA would expect to see some sort of dose response over this magnitude of concentration difference. In sum, a POSA would view the results in Figure 22 for H53D [sic] (+39+69) as reflecting technical defects in the underlying exon 53 assay, and therefore give it little weight.

23. A POSA would understand from statements in the UWA Specification that H51A(+61+90) and H51A(+66+95) were the strongest inducers of exon 51 skipping, and that H52A(+12+41) and H52A(+17+37) showed the strongest exon 52 skipping. '851 Patent at 62:39-63:49 and Tables 37 and 38. Yet, Figure 22 does not show the data for the strongest skippers H51A(+61+90), H51A(+66+95) or H52A(+12+41). Instead, Figure 22 includes lanes for AONs the UWA Specification indicates “needs re-testing” (H51A(+111+134)), “skipping” (H51A(+66+90)) and three reported as “no skipping” (H52A(-07+14), H52A(+93+112) and H52D(+05-15)).

Table 1. Wilton PCT '057 Experimental Results.

Adapted from Wilton PCT '057 Tables 37 and 38

Bold indicates run in Figure 22 gel

SEQ ID NO.	AON	Ability to Induce Exon Skipping
175	H51A(-01+25)	Faint skipping
177	H51D(+16-07)	Skipping at 300 nM

SEQ ID NO.	AON	Ability to Induce Exon Skipping
178	H51A(+111+134)	Needs re-testing
179	H51A(+61+90)	Very strong skipping
180	H51A(+66+90)	Skipping
181	H51D(+66+95)	Very strong skipping
182	H51D(+08-17)	No skipping
183	H51A/D (+08-17) & (-15+?)	No skipping
184	H51A(+175+195)	No skipping
185	H51A(+199+220)	No skipping
186	H52A(-07+14)	No skipping
187	H52A(+12+41)	Very strong skipping
188	H52A(+17+37)	Skipping to 50 nM
189	H52A(+93+112)	No skipping
190	H52D(+05-15)	No skipping

24. In scientific publications, it is typical to include a clean, sharp image available to the authors and a best practice to include detailed methodological descriptions and other means by which other researchers can assess the reproducibility or quantitative significance of the data. It is highly atypical to include inconclusive results, or an unlabeled and poor-quality image like Figure 22. Thus, when Figure 22 is viewed as a whole and in the context of the UWA Specification, including the selection of samples run, it reinforces that a POSA would view the data as preliminary—potentially so preliminary that the inventors did not have time to reproduce the results or at least re-run their best AONs for these three exons on one clean gel.

25. Contrary to Dr. Dowdy’s assertion, Figure 22 does not confirm the results reported in Table 39 for H53A(+39+69). Table 39 specifically reports “strong skipping to 50 nM” and the UWA Specification states H53A(+39+69) and other exon 53 AONs were tested at five

concentrations. There are only four lanes in Figure 22, and they are not labelled. Thus, Figure 22 does not confirm the inventors observed “strong skipping to 50 nM” as reported in Table 39. *Cf.* Dowdy Rebuttal ¶¶ 461, 468. While a POSA would need to accept the information in Table 39 at face value, he or she would view those results as preliminary as well after reviewing the UWA Specification as a whole and in the context of the general consensus and understanding in the field that exon skipping was unpredictable.

26. Dr. Dowdy appears to believe that the potential discrepancy in the UWA Specification on the concentrations tested and issues with Figure 22 would have essentially no significance to a POSA. Dowdy Rebuttal ¶ 461. I disagree. Understanding what controls were used and concentrations tested is an essential and basic requirement for understanding whether the observed results reflect true skipping activity or are rather merely part of the noise inherent in the experiment. The UWA Specification falls short on this and other such essential and basic requirements, such as explaining experimental methods in sufficient detail so that their results can be understood and if necessary replicated. The lack of clarity, lack of specified and labeled controls, lack of detailed methodological description, and inconsistencies all undermine the scientific method and all would cause a POSA to question the quality of the scientific evidence presented in the UWA Specification.

27. Dr. Dowdy also misstates the number of overlapping exon 53 AONs made and tested by the inventors: there were five, spanning +7 to +69, not four spanning +23 to +69 bases downstream of the exon 53 acceptor site. *See* ‘851 Patent, Table 39 *cf.* Dowdy Rebuttal ¶¶ 463, 467. Dr. Dowdy also makes no attempt to explain why he set the 5’ end of the “hot spot” at +23 when “no skipping” was reported for an AON targeting H53A(+7+29). Nor does Dr. Dowdy to explain why he set the 3’ end of the “hot spot” to +69 when the inventors did not test an overlapping

AON that extended 3' of +69. Nor does Dr. Dowdy explain why the target region of H53A(+150+176), which showed “very faint skipping to 50 nM” according to Table 39, would not be considered a “hot spot” under his flexible definition based on any degree of observable exon 53 activity. Dr. Dowdy’s selection of +23 and +69 as the boundaries of the “hot spot” is either arbitrary or tainted by hindsight bias, or both. A POSA would not have concluded from the UWA Specification that the inventors had invented a “hot spot” or amenable region of +23 to +69.

28. I disagree with Dr. Dowdy’s statement that the UWA Specification “describes a group of overlapping ASOs directed to a hot spot of human exon 53 that spans nucleotides +23 to +69.” Dowdy Rebuttal ¶ 467. Dr. Dowdy’s use of “directed to” implies that the inventors designed the exon 53 AONs in the UWA Specification with the foreknowledge that they would induce skipping. That is obviously counterfactual in view of the lack of predictability in the art of exon skipping as discussed in my Opening Report and Rebuttal Report. Rather, a POSA would understand that the inventors designed a series of AONs spanning large portions of exon 53 in hopes of finding some through trial-and-error experimentation that would induce skipping. Contrary to Dr. Dowdy’s assertion, the UWA Specification simply does not “reveal” that the inventors “expected [the AONs] to induce exon 53 skipping.” Dowdy Rebuttal ¶ 467. A POSA would understand based on the unpredictability of the art at the time of filing that the inventors would not have had an expectation that any of the exon 53 AONs they designed, whether as of the priority date or afterwards, would induce skipping before testing them empirically.

29. Indeed, designing a series of AONs targeting large portions of an exon, like the exon 53 AONs described in the UWA Specification, was a typical first step (of many) to search for and identify AONs with skipping activity for a previously-unexplored exon. I note that prior to the publication of the UWA Specification, the inventors had not published any work concerning

exon 53. Further, a POSA would have been aware that, as of late October 2004, the Wilton laboratory was reported to be working on designing AONs targeting exons 4, 8, 9, 15, 16, 19 and 20, 31, 33 and 35 that are towards the 5' portion of the dystrophin gene. *See* Muntoni et al., *Neuromuscular Disorders* 2005; 15:450-57 at 453-54. This would also reinforce to a POSA that the work reported in the UWA Specification on exon 53 was preliminary.

2. There is No Disclosure that Any 12 Consecutive Bases of SEQ ID NO: 195 (H53A(+23+47)) is Characteristic of Exon 53 Skipping AONs

30. Dr. Dowdy's opinion with respect to the "at least 12 consecutive bases of SEQ ID NO: 195" limitation of the UWA Patent claims, is similarly arbitrary or plagued by hindsight bias. Nowhere does the UWA Specification reflect any possession, recognition, or appreciation that any 12 consecutive bases of SEQ ID NO: 195, which is H53A(+23+47), is a common structural feature of all exon 53 skipping AONs.

31. The UWA Specification mentions the phrase "12 bases" once and in the context of discussing AONs for "some targets such as exon 19"—not exon 53—and states that such AONs were "not as efficient[] as longer (20-31 bases) oligonucleotides" at inducing skipping:

Furthermore, the inventors have discovered that size or length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules. With some targets such as exon 19, antisense oligonucleotides as short as 12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides. In some other targets, such as murine dystrophin exon 23, antisense oligonucleotides only 17 residues long were able to induce more efficient skipping than another overlapping compound of 25 nucleotides.

'851 Patent at 23:60-24:3.⁵

⁵ A POSA would have been aware that the discovery by the inventors that a 12 base AON was able to induce skipping was in fact limited to exon 19, and only in immortalized mouse cells with a mutation in exon 23, as described in Errington et al., *J. Gene Med.* 5: 518-527 (2003) at 523 (describing testing of one 12 base AON, HM19A(+46+57), in *mdx* cells). An earlier publication

32. Thus, the UWA Specification fails to discuss “12 bases:” (i) as a “base sequence” that is part of an AON; (ii) in connection with exon 53; or (iii) in connection with SEQ ID NO: 195. In fact, the UWA Specification does not disclose a 12 base AON *at all*; the shortest AON disclosed is the 17 base SEQ ID NO: 16 (C16D(+06-11)).

33. From reading the UWA Specification, a POSA would have no inkling that the inventors had discovered that any 12 consecutive bases of SEQ ID NO: 195/H53A(+23+47) was a structural feature common to all exon 53 skipping AONs—if it is indeed such a feature. In particular, 12 consecutive bases of SEQ ID NO: 195 is *not* a structural feature of any of the other exon 53 AONs disclosed in the UWA Specification. The only AON reported to induce skipping in Table 39 that has 12 consecutive bases of H53A(+23+47) is H53A(+23+47) itself. And, 12 consecutive bases is not a structural feature of the three AONs that were reported to have more skipping activity than H53A(+23+47) in Table 39. Thus, the UWA Specification informs a POSA that 12 consecutive bases of H53A(+23+47) is *not* a structural feature common to all exon 53 skipping AONs.

34. Dr. Dowdy criticizes me for focusing on the 12 consecutive bases of H53A(+23+47) limitation of the UWA Patent claims, purportedly taking it out of context. Dowdy Rebuttal ¶ 473. I disagree that I have taken this claim limitation out of context. Further, my consideration of this limitation is appropriate. Notably, the three AONs that were reported to have more skipping activity than H53A(+23+47) in Table 39 each have the other structural features that, according to Dr. Dowdy, “collectively confer the claimed function of inducing exon 53 skipping,” namely, they are antisense oligonucleotides (Dowdy feature no. 1), 20 to 31 bases in length

from the Wilton laboratory reported that no detectable skipping was observed by 14 base AONs targeting M23D(-2-15) and M23D(-5-18). Mann et al., J. Gene Med 4: 644-654 (2002) at 650.

(Dowdy feature no. 2), “100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA” (Dowdy feature no. 3); the morpholino versions of which would have uracil bases instead of thymine bases (Dowdy feature nos. 5-6). Dowdy ¶ 473. Thus, the 12 consecutive bases of H53A(+23+47) limitation is critical to distinguishing the claimed genus of AONs from other exon 53 AONs disclosed in the UWA Specification.

35. These three other AONs also target Dr. Dowdy’s purported “hot spot” but are outside the scope of the claims. This illustrates that Dr. Dowdy’s discussion of how the inventors purportedly identified a “hot spot” or amenable region within exon 53 is a misdirection that conflates identifying a “hot spot” with identifying every potential AON targeting that region, much less every such AON having exon 53 skipping activity. Even assuming that the inventors did identify a “hot spot” or amenable region within exon 53 (and that a POSA would have understood this from the UWA Specification—which they would not have) it does not follow that the inventors contemporaneously conceived, possessed, recognized, or appreciated each of the AONs that fall within the scope of the UWA Patents’ claims, particularly given that its strongest performing AON H53A(+39+69) falls outside the scope of the claims. Likewise, it does not follow that the inventors conceived, possessed, recognized, or appreciated that a subset of AONs including at least 12 consecutive bases of SEQ ID 195 within that purported “hot spot” had any particular structural feature that made them particularly amenable to exon 53 skipping.

3. There is No Discussion of 20 to 31 Bases as an Appropriate Range of Lengths for Exon 53 Skipping AONs

36. Dr. Dowdy states that “[o]nce the exon 53 hot spot was identified, the inventors of the Wilton Patents would have been able to draw from their extensive experience and identify an appropriate range of ASO length for inducing exon 53 skipping,” thereby arriving at the “20 to 31 bases” recited in the UWA Patent claims. Dowdy Rebuttal ¶ 465 (emphasis added). But

this puts the chicken before the egg. The inventors necessarily selected the length of the exon 53 AONs identified in the specification *before* identifying any purported hot spot by testing those AONs. Therefore, a POSA would understand the inventors merely hoped that the length of their exon 53 AONs were “appropriate” rather than recognizing or appreciating that range prior to testing the AONs. While the UWA Specification states that “[p]referably the length of the antisense molecule is between 17 to 30 nucleotides in length” (’851 Patent at 26:2-3), the inventors did not explain why they departed from this preferred range in designing AONs for exon 53.

37. I also disagree with Dr. Dowdy’s opinion that the UWA Specification “reveals that the inventors had a ‘definite and permanent’ idea of ASOs that are 20 to 31 bases in length, directed to this discrete [+23+69] region, and expected to induce exon 53 skipping” (Dowdy Rebuttal ¶ 467) at least because the UWA Specification does not disclose an AON shorter than 25 bases against the +23+69 region. Thus, a POSA would not understand from the UWA Specification that the inventors had invented any AONs shorter than 25 bases that could induce exon 53 skipping. Notably, the longest AON, the 31 base H53A(+39+69), was reportedly the strongest skipper, with shorter, 25 base AONs that were entirely within that same region (H53A(+45+69) and H53A(+39+62)) showed only faint skipping. If anything, these preliminary results would have indicated to a POSA that a 31mer AON might work better for exon 53 than shorter, 25mer AONs. Further, the sole 20mer tested, H53A(+07+26), was reported as “no skipping” despite overlapping with a 25mer, H53A(+23+47), that induced very faint skipping.

38. Upon reviewing the preliminary results reported in Table 39 and the UWA Specification as a whole, a POSA would not understand the inventors to have had an expectation that AONs of 20 bases would induce exon 53 skipping, or understand that the inventors had

invented AONs of less than 25 bases that were capable of inducing exon 53 skipping, regardless of annealing site. *Cf.* Dowdy Rebuttal ¶ 472.

C. A POSA Would Have Concluded the Inventors Possessed Only the AONs Disclosed in the UWA Specification

39. As I have described in my Opening Report and Rebuttal Report, designing AONs for exon skipping for Duchenne Muscular Dystrophy was unpredictable in 2005 and remains unpredictable to this day, including for exon 53. The UWA Specification added some preliminary data and knowledge to the field, but that is all. In fact, I am unaware of any researcher in the field actually relying on the teachings of the UWA Specification to design AONs. In my experience, researchers and POSAs in the field relied on their own work and peer-reviewed academic publications over patent disclosures which are not subject to peer review because of the unpredictability of exon skipping and the need to empirically test and validate AON sequences.

40. A POSA would not have viewed one unlabeled gel and a subjective description of skipping, which is the sum total of the disclosure in the UWA Specification with respect to exon 53, to have reduced the degree of predictability in the field. A POSA reviewing the UWA Specification in June 2005 would not have understood the inventors to have invented the structural features recited in the claims that were submitted in 2017 and which Dr. Dowdy claims collectively confer exon 53 skipping activity. Indeed, those structural features are *not* common to all of the exon 53 AONs reported to have skipping activity according to the UWA Specification. Therefore, in my opinion, the inventors did not invent the claims of the UWA Patents as of the filing date of Wilton PCT '057.

III. RESERVATION OF RIGHTS AND TRIAL EXHIBITS

41. I reserve the right to amend or supplement my opinions once I review any new/additional information and/or other new/additional documents or information subsequently produced by Sarepta, UWA, or any other party, including via rebuttal expert reports of Sarepta's and UWA's experts.

42. At trial I may use visual aids and demonstratives to show the bases for my opinions, such as photographs, drawings, excerpts from documents and materials I considered, videos, and animations. I reserve the right to utilize these at trial.

Exhibit 19

**IN THE UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE**

**NIPPON SHINYAKU CO., LTD.,
Plaintiff,**

v.

**SAREPTA THERAPEUTICS, INC.,
Defendant.**

C.A. No. 21-1015 (GBW)

**SAREPTA THERAPEUTICS, INC. and
THE UNIVERSITY OF WESTERN
AUSTRALIA, Defendant and Counter-
Plaintiff**

v.

**NIPPON SHINYAKU CO., LTD. and
NS PHARMA, INC., Plaintiff and
Counter-Defendants.**

EXPERT REBUTTAL REPORT OF DR. MATTHEW J.A. WOOD

October 11, 2023



Matthew J.A. Wood, F. Med. Sci., MA, D.Phil

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I. INTRODUCTION AND ASSIGNMENT

1. I was retained by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) in Nippon Shinyaku Co. Ltd. v. Sarepta Therapeutics, Inc. as an independent technical expert. In connection with my engagement, I issued a report on September 8, 2023 in which I was asked to provide an opinion concerning the state of the art of exon skipping therapies for treatment of Duchenne muscular dystrophy (“DMD”) and what a person of ordinary skill in the art (“POSA”) would have understood the inventors of the asserted patents to have invented based on the Specification (the “Opening Report”).

2. On September 8, 2023, counsel for Sarepta Therapeutics, Inc (“Sarepta”) and the University of Western Australia (“UWA”) served expert reports, including the expert report of Dr. Steve F. Dowdy, Ph.D. (the “Dowdy Report”). I was asked to prepare this rebuttal report to review and respond to the Dowdy Report.¹ I have reviewed only publicly available information in doing so.

3. I understand that NS alleges that Sarepta Therapeutics, Inc. (“Sarepta”) infringes several of NS’s patents and that Sarepta alleges that NS infringes three patents owned by the University of Western Australia (“UWA”) and exclusively licensed by Sarepta. More specifically, I understand NS has asserted claims 1-3 of U.S. Patent No. 10,385,092 (“the ’092 patent”); claims 1-2 of U.S. Patent No. 10,407,461 (“the ’461 patent”); claims 1-2 of U.S. Patent No. 10,487,106 (“the ’106 patent”); claims 1-12 of U.S. Patent No. 10,647,741 (“the ’741 patent”); claims 1-4 of

¹ I received a copy of the Dowdy Report in redacted form. I understand the redactions covered Sarepta confidential information.

U.S. Patent No. 10,662,217 (“the ’217 patent”); and claims 1-4 and 6-9 of U.S. Patent No. 10,683,322 (“the ’322 patent”) (collectively, the “NS Patents”) against Sarepta and UWA.

4. I understand from counsel for NS (“counsel”) that I may be provided additional information as this case proceeds. Accordingly, I may need to change or augment my analysis and opinions in light of any new information or evidence that is presented after this Report. I expressly reserve the right to do so.

5. My consulting rate for this case is £650 GBP per hour. My compensation is not related to the outcome of this action, and I have no financial interest in the outcome of this case.

II. QUALIFICATIONS

6. A discussion of my qualifications to testify about my opinions herein, was provided in my Opening Report.

III. BACKGROUND OF ANALYSIS

7. In considering and forming my opinion, I have reviewed and analyzed the information and materials identified in this Report. As stated, a list of the materials I reviewed in preparation of this report is attached as Exhibit 1.

8. I am not an attorney and do not have formal training in the law regarding patents. This section presents my understanding of currently applicable legal principles, explained to me by counsel, which I have used in forming my opinions.

A. Person of Ordinary Skill in the Art

9. I am informed that a POSA is a hypothetical person skilled in the relevant art, not a judge, not a layperson, not a person skilled in the remote arts, and not a genius in the relevant art. Relevant factors in determining the level of ordinary skill in the art include the educational level of the inventors of the patent in suit and of those working in the field at the relevant time.

Other relevant considerations include various prior art approaches employed in the art, types of problems encountered in the art, the rapidity with which innovations are made, and the sophistication of the technology involved.

10. I understand that NS and Sarepta have generally offered similar definitions of a POSA for the NS Patents. Dowdy Rpt. ¶¶ 19-20. For purposes of this report, I have applied Dr. Dowdy's definitions of a POSA for the NS Patents. However, if NS's definition were adopted, this does not affect my opinions.

IV. SCOPE AND CONTENT OF THE PRIOR ART AS OF AUGUST 2011

A. There Were No Recognized "Hot Spots" Within Exon 53 As of August 2011.

11. Dr. Dowdy opines that "by August 31, 2011, the hot spot within exon 53 had been identified and repeatedly verified." Dowdy Rpt. ¶ 110. I disagree. Dr. Dowdy arrives at this opinion by selectively reviewing the art and cherry-picking results to determine such a "hot spot." *Id.* ¶¶ 91-109. As I describe below, as of August 31, 2011, there was no recognized "hot spot" within exon 53 for targeting with an AON to induce skipping of exon 53. In my experience, "hot spots" are vanishingly rare, and to my knowledge do not exist within the human dystrophin gene. Thus, while I adopt Dr. Dowdy's term, I do not agree with the underlying premise that a "hot spot" within exon 53 in fact exists, much less was recognized in 2005 by Dr. Wilton or others.

1. State of the Art as of June 2005

12. As of 2005, there were only a few groups working to develop antisense oligonucleotides ("AONs") for treatment of Duchenne Muscular Dystrophy. Several research groups had joined consortiums based largely on geography – Netherlands/Belgium and United Kingdom – and there were also groups working in Japan. *See* Muntoni et al., Neuromuscular

Disorders 15:450-57 (2005) (“Muntoni 2005”), at 456. I was a member of the UK consortium, as was Steve Wilton. As of late 2004, UK researchers’ work on AONs targeting exons 51 and 53 was well underway, with clinical trials contemplated. At that time, Dr. Wilton’s focus was on exons from the 5’ end of the dystrophin gene, *i.e.* exons 35 and below. *See id.* at 453-454.

13. Dr. Dowdy discusses publications from groups working at Kobe University and Leiden University reporting on exon 53 skipping AONs prior to June 2005. Dowdy Rpt. ¶¶ 92-96 (discussing Aartsma-Rus et al., *Neuromuscular Disorders* 12:S71-S77 (2002) (“Aartsma-Rus 2002”), U.S. Patent Number 6,653,467 from Matsuo et al. (“Matsuo US ’467”), and EP Publication No. 1568769 (“Matsuo EP ’769”), an English version of PCT Patent Publication No. WO2004/048570 by Matsuo et al.). He opines that “the studies from these two groups were somewhat contradictory” because Matsuo’s ethylene-bridged nucleic acid (“ENA”) AO71, targeting the coordinates (+129+146), was reported to induce exon skipping while Aartsma-Rus’ h53AON2, targeting bases (+128+145) was reported to not induce exon 53 skipping—despite being shifted just one base. Dowdy Rpt. ¶ 96. I disagree with Dr. Dowdy’s characterization.

14. First, the results for Matsuo’s AO71 showed no bands at all. Matsuo EP ’769, at Fig. 18. Accordingly, the description of this figure cited by Dr. Dowdy is clearly erroneous, and no conclusions can be drawn regarding AO71’s exon 53 skipping activity. Even if I were to assume that Matsuo had undisclosed data showing that AO71 induced exon skipping, I would disagree that this would be contradictory to Aartsma-Rus’ data. Rather, it could be an example of the variability that is well-known in the field, either laboratory-to-laboratory or experiment-to-experiment. Alternatively, it could be an example of AON to AON variability where shifting the AON target site by one base has a significant effect on exon skipping activity. Many factors, including AON backbone chemistry, AON length, AON concentration, cell type tested,

incubation period, transfection method, use of delivery technology (e.g., endosomal escape or nuclear localization), and the inherent variability of primary cell cultures like primary myotubes and human skeletal muscle cells, may confound comparisons of results from different laboratories, even of AONs with the same sequence and backbone. *See also* Opening Report ¶¶ 87-89. Dr. Dowdy acknowledges that these differences can affect AON delivery and exon skipping activity. Dowdy Rpt. ¶¶ 640-641.

15. Due to the unpredictability of exon skipping and variability in assay conditions used by different laboratories, and in different experiments from the same laboratory, it was and remains common for laboratories to make and test AONs having sequences published by other laboratories (and often variations thereof) to use as comparators, rather than merely relying on the published data. This is a way to account for inter-laboratory variability and also confirms the robustness of the skipping effect. It allows for a better assessment of the criticality of the target site and whether differences in results are primarily experimental noise.

16. Thus, Aartsma-Rus 2002, Matsuo US '467, and Matsuo EP '769 illustrate the difficulties a person of ordinary skill in the art ("POSA") would have had in drawing general conclusions about what regions in exon 53 to target based on experimental data from different laboratories.

17. Second, as I explained in my opening report, changing an AON by even a single base can change its ability or effectiveness to induce exon skipping. Opening Report ¶¶ 94, 135-137. Similarly, shifting an AON in the 5' or 3'-direction even by a single base can also change its ability or effectiveness to induce exon skipping. That is why the general consensus in the field as of 2004 and today is that the exon skipping potential of an AON must be assessed empirically.

18. Thus, I generally agree with Dr. Dowdy that the limited data in Aartsma-Rus 2002 and the Matsuo references were insufficient to support the recognition of a particular region within exon 53 that would be amenable for exon skipping. Dowdy Rpt. ¶ 96. Where Dr. Dowdy and I diverge is the impact of the publication of PCT Patent Publication No. WO2006/000057 by Wilton et al., (“Wilton PCT ’057”) on January 5, 2006. In my opinion, Wilton PCT ’057 was preliminary, not “pivotal” and did not identify a “hot spot” spanning +23 to +69 of exon 53. *Cf.* Dowdy Rpt. ¶¶ 97, 99.

2. Wilton ’057: January 2006

19. Wilton PCT ’057 published on January 5, 2006. I understand from counsel that Wilton PCT ’057 is the publication of the June 28, 2005 PCT to which the patents that Sarepta and UWA are asserting against NS—U.S. Patent No. 9,994,851 (“the ’851 Patent”), U.S. Patent No. 10,227,590 (“the ’590 Patent”), and U.S. Patent No. 10,266,827 (“the ’827 Patent”)—claim priority. I also understand that Wilton PCT ’057 is substantively similar to the ’851 Patent, ’590 Patent, and ’827 Patent. Accordingly, my analysis of the UWA Patents in my Opening Report is also applicable here. *See* Opening Report § V.

20. Wilton PCT ’057 discloses 211 AONs for inducing exon skipping in one of exons 3 to 8, 10 to 16, 19 to 40, 42 to 44, 46, 47, and 50 to 53 of the human dystrophin pre-mRNA Wilton PCT ’057 at 6:6-8; 11-16. Of the 211 disclosed AONs, 12 target exon 53. *Id.* at 16 (SEQ ID Nos: 191-202). Of these 12 AONs, 7 target purely exonic sequences and 5 target intronic and exonic sequences—2 at the acceptor splice site, and 3 at the donor splice site. *Id.*

21. The specification notes that “[a]ttempts by the inventors to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most

effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies.” *Id.* at 35:2-6. In other words, the inventors admit that they had failed to identify rules and principles that could be rationally applied to identifying effective AONs, and thus had to rely on empirical testing.

22. Eleven of the 12 exon 53 AONs were synthesized as 2’-O-methyl AONs and tested for exon skipping activity by transfecting each AO in primary human myoblasts; the twelfth was “not made yet.” *Id.* at 35:15-17, 24-29, Table 39. The inventors reported that the eleven AONs “showed varying ability to induce exon 53 skipping,” and that H53A(+39+69) “induced the strongest exon 53 skipping.” *Id.* at 62:13-15. Wilton PCT ’057 Table 39 (reproduced in Table 1 part below) provides the results of these experiments.

Table 1. Wilton PCT ’057 Experimental Results.
Adapted from Wilton PCT ’057 Table 39

SEQ ID NO.	AON	Ability to Induce Exon Skipping
191	H53A(+45+69)	Faint skipping at 50 nM
192	H53A(+39+62)	Faint skipping at 50 nM
193	H53A(+39+69)	Strong skipping to 50 nM
194	H53D(+14-07)	Very faint skipping to 50 nM
195	H53A(+23+47)	Very faint skipping to 50 nM
196	H53A(+150+176)	Very faint skipping to 50 nM
197	H53D(+20-05)	Not made yet
198	H53D(+09-18)	Faint at 600 nM
199	H53A(-12+10)	No Skipping
200	H53A(-07+18)	No Skipping
201	H53A(+07+26)	No Skipping
202	H53A(+124+145)	No Skipping

23. There is no disclosure in the specification of experimental details, or confirmation that experimental conditions such as AON concentrations, incubations times, and transfection agents were identical for the results reported in Table 39 of the specification. Indeed, the specification describes three different delivery methods: cationic lipoplexes, mixtures of AONs, or cationic liposome preparations. Wilton PCT '057 at 35:29-36:2. Nor is there any disclosure of whether the results reported for the exon 53 AONs were reproducible, or how many replicates were done. In sum, a POSA reading the specification would not know how skipping was assessed or analyzed, whether bands were quantified, experiments repeated, or statistical analyses conducted. Given the scant information on experimental parameters that are known to affect the results observed, a POSA would view Wilton PCT '057 as describing results that were preliminary, not conclusive.

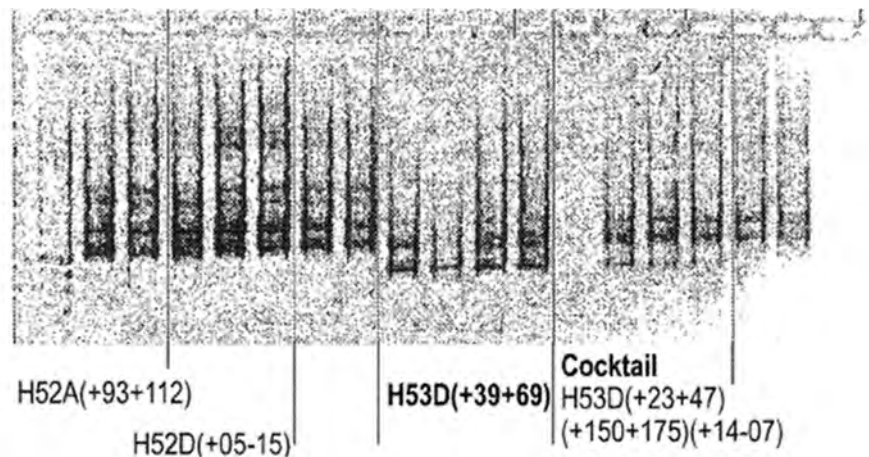
24. Dr. Dowdy opines from these results that it “is readily apparent that some of the ASOs that were reported to induce exon 53 skipping are clustered between the 23rd to the 69th nucleotides of human exon 53” and that this demonstrates “for the first time a discrete region within exon 53 that is amenable for exon skipping (sometimes referred to as the ‘hot spot’).” Dowdy Rpt. ¶ 99. I disagree. No POSA would have relied on the scant and clearly preliminary data of Wilton PCT '057 to conclude there is a “hot spot” from +23 to +69 of exon 53. At most, Wilton PCT '057 showed that one AON, H53A(+39+69), had sufficient skipping activity to make it a reasonable choice for further investigation from among the AONs tested.

25. Wilton PCT '057 characterizes the ability of each AON to induce exon skipping as “very faint,” “faint,” or “strong.”² A POSA would view these subjective and apparently

² Wilton PCT '057 provides a definition of an “efficient” antisense molecule, as “one that induces strong and sustained exon skipping at transfection concentrations in the order of 300 nM

arbitrary descriptions with skepticism. There is no explanation as to the criteria used to assign these descriptions, or whether they were assigned after multiple experiments or just one. It is also unclear whether “skipping to,” or “skipping at” a particular concentration is an intentional distinction that describes different results or merely sloppy drafting.

26. Figure 22 of the UWA Patents provides the only objective data concerning exon 53 skipping. It is a gel showing the exon 53 skipping induced by H53A(+39+69), amongst other samples:



27. As I explained in my Opening Report, Figure 22 provides little additional insight into what the inventors had discovered about exon 53 skipping AONs generally, or H53A(+39+69) specifically, as of the priority date. There are also internal inconsistencies that cast doubt on the reliability of individual statements in the specification. For example, the specification states that Figure 22 shows that H53A(+39+69) “was able to induce exon 53 skipping at 5, 100, 300 and 600 nM.” Wilton PCT '057 at 62:6-7. However, Table 39 of the specification reports that H53A(+39+69) induced “strong skipping to 50 nM.” *Id.* at 62 (emphasis added). It is unclear

or less” but does not use that term to characterize any of the exon 53 AONs tested. Wilton PCT '057 at 36:21-23.

whether this is an intentional description of different experimental results or a typographical error; the discrepancy cannot be resolved by reviewing Figure 22 because the lanes in the figure for “H53D(+39+69)” [sic] are not labeled with the concentrations tested.

28. As I explained in my opening report, it is not possible to predict whether two or more AONs having overlapping bases will uniformly induce exon skipping (or induce exon skipping at all) merely as a result of the overlapping bases. Opening Report ¶¶ 135-136. At the extreme, I am aware of examples where only a single nucleotide difference in sequence affects the exon skipping of an AON. *See id.* ¶ 139. Accordingly, I disagree with Dr. Dowdy’s opinion that it is “readily apparent” that the exon 53-skipping AONs are “clustered” in the (+23+69) region. A POSA would not have identified a “hot spot” in exon 53 based on the scant results from the four AONs the Wilton laboratory tested within that region—two of which reported as “faint skipping” and one “very faint.” In my opinion, the “faint” and “very faint” results refutes Dr. Dowdy’s claim that Wilton PCT ’057 identifies a “hot spot.”

29. I also disagree that a POSA would have viewed the results as describing a “cluster” of exon skipping AONs, much less a “readily apparent” cluster extending to +23. H53A(+23+47) showed only “very faint skipping.” It overlaps with H53A(+39+69), the only AON to induce “strong skipping,” by only 9 bases—less than half of the size of the H53A(+23+47) 25mer and less than one third the size of the H53A(+39+69) 31mer. In view of the “very faint skipping” and small degree of overlap, the 5’ end of H53A(+23+47) would not have been viewed as part of a “cluster” with the “strong” and “faint” skippers targeting the (+39,+69) region. If anything, the “no skipping” result reported for H53A(+7+26) would indicate that the region between the +23 and +26 bases of exon 53 was *not* part of any “hot spot.”

30. Moreover, Dr. Dowdy's assertion that Wilton PCT '057 identifies a "hot spot" ignores that only a small portion of exon 53 is tested, and with only a small number of AONs. There are hundreds of millions of possible AONs within the putative +23 to +69 "hot spot," of varying length, backbone chemistry, and degree of complementarity, and Dr. Wilton tested only two AONs. It is not scientifically plausible that a POSA would develop an expectation that other AONs targeting this region would cause (or would be more likely to cause) exon skipping. Cf. Dowdy Rpt. ¶ 101. The only conclusion that a POSA would have drawn regarding exon 53 skipping AONs based on the limited data and vague and subjective description of "skipping" in Wilton PCT '057 was that H53A(+39+69) had the most activity of the AONs tested. It is the only one that might possibly meet the definition of "efficient" ("induces strong and sustained exon skipping at transfection concentrations in the order of 300 nM or less") and thus be a potential starting point for further refinement as taught by the inventors. Wilton PCT '057 at 36:19-24. However, the data and information in the Wilton PCT '057 is so scant that even the positive result for H53A(+39+69) would have been viewed with skepticism by a POSA.

3. Post-2006 Publications

31. The number of research groups studying AONs for treating DMD did not significantly increase from 2005 to 2008 to 2012. This is illustrated by the number of participants on the ENMC workshops on antisense oligonucleotides in DMD over the years. In late 2004, twenty-six participants including parents, scientists, industry representatives, and clinicians participated in the 128th ENMC International Workshop. Muntoni 2005 at 1. The next ENMC workshop on this topic was held in late 2007, and thirty-one participants attended, including myself. Muntoni et al., *Neuromuscular Disorders* 18:268-275 (2008) ("Muntoni

2008”). The next ENMC workshop in this field was held in late 2012, and twenty-seven participants attended. Aartsma-Rus and Muntoni, *Neuromuscular Disorders* 23:934-944 (2013).

32. In my opinion, publications post-dating Wilton PCT ’057 demonstrate that there was no recognition in the art that the Wilton laboratory had identified a discrete region within exon 53 that is amenable for exon skipping that spanned the 23rd to 69th bases of exon 53. *Cf.* Dowdy Rpt. ¶ 99. This includes publications from the Wilton laboratory itself, indicating they had not recognized the existence of such a region either, as well as Royal Holloway College and Sarepta. The publications discussed below thus represent a significant fraction of the half-dozen or so academic research groups and biotechnology companies conducting AON research for DMD in the 2006 to 2011 timeframe.

a. Harding: January 2007

33. In January 2007, the Wilton laboratory published a paper reporting the results of testing AONs that were identified in Wilton PCT ’057.³ Harding et al., *Mol. Ther.* 15(1):157-66 (2007) (“Harding 2007”). The results reported in Harding 2007 are shown in Table 2 below. The “skipping efficiency” is reported as “-,” “+/-,” “+,” or “++”. *Id.* This reporting nomenclature is not defined. Therefore, a POSA would not have been able to draw conclusions about the absolute skipping efficiency of any individual AON, and would have found it difficult to draw any conclusions on the relative skipping efficiency of the AONs that exhibited some degree of activity.

Table 2. Summary of the results reported in Harding 2007.

³ Harding 2007 does not provide results for H53A(+150+176), identified in Wilton PCT ’057 as SEQ ID NO: 196; rather, it reports the results of testing H53A(+151+175). Harding 2007 at Table 2.

AON	Skipping efficiency
H53A(-07+18)	-
H53A(-12+10)	-
H53A(+23+47)	+/-
H53A(+39+62)	+/-
H53A(+39+69)	++
H53A(+45+69)	+
H53A(+124+145)	-
H53A(+151+175)	+/-
H53D(+09-18)	+/-
H53D(+14-07)	+/-

34. It is also difficult to reconcile the results reported in Harding 2007 with the results reported in Wilton PCT '057. For example, Wilton PCT '057 reports that H53A(+39+62) (SEQ ID NO: 192) and H53A(+45+69) (SEQ ID NO: 193) each induced “faint skipping at 50 nM.” See Table 1, *supra*. However, Harding 2007 reports these two AONs, having exactly the same sequence and same 2'-O-methyl backbone chemistry as in Wilton PCT '057, induced different levels of skipping: “+/-” and “+,” respectively. See Table 2, *supra*. Further, the data provided in Harding 2007 shows these two AONs induce very weak skipping at 600 nM, without even a trace of “faint skipping” to be seen at 50 nM. Harding 2007, at 164 and Fig. 4b and 4c. The data in Harding 2007 for H53A(+39+69) is roughly consistent with the results reported in Table 39 of Wilton PCT '057 (“strong skipping at 50 nM”). However, it is difficult to compare the gels shown in Harding Fig. 4d and Wilton PCT '057 Fig. 22 because the lanes are not labeled in the latter.

35. Due to the lack of information in Wilton PCT '057, it is unclear whether Harding 2007 describes results from the same experiments or uses the same parameters as Wilton PCT '057, or describes results of additional experiments using different parameters. Regardless, these near-contemporaneous yet inconsistent results from the same laboratory concerning some of the same exon 53 AONs cast doubt on the disclosures in Wilton PCT '057. However, the conclusion that H53A(+39+69) had the most activity of the AONs tested was consistent between the two publications. Harding 2007 even expressly states that the amenable site to redirect dystrophin splicing within exon 53 was +39+69. *Id.* at 164.

36. In my opinion, Harding 2007 shows only that there is a single AON in exon 53 that appears to have some good activity. There is insufficient data to conclude that this region is a “hot spot” because shorter AONs between +39 +69 had only very weak activity. Certainly there is no indication in either Wilton PCT '057 or Harding 2007 that AONs binding upstream of +39 would have good skipping activity.”

37. Harding 2007 shows that the Wilton laboratory focused on the surrounding region encompassed by their strongest skipper—H53A(+39+69)—to assess the effect of length. *Id.* at 158, Figure 4. Their results indicated that the 31 base AON spanning the +39 to +69 region was far more active than shorter AONs within the that region. *Id.* From this a POSA would have understood that AON length may be an important determining factor for exon 53 in particular, although there is insufficient data in Harding 2007 to conclude that length was more important than target sequence or vice versa.

b. Wilton: July 2007

38. In July 2007 (seven months after publishing Harding 2007), the Wilton laboratory published another paper reporting the results of testing some AONs that were identified in

Wilton PCT '057. Wilton et al., Mol. Ther. 15(7):1288-1296 (2007) ("Wilton 2007"). The only AON for exon 53 discussed was H53A(+39+69). Wilton 2007, Table 1 and Fig. 2(a). This indicates that the Wilton laboratory continued to focus on the H53A(+39+69) AON, not the other AONs tested in Wilton PCT '057.

c. Popplewell (Royal Holloway University): January 2009

39. In January 2009, George Dickson's group at Royal Holloway University of London published a paper reporting the design of phosphorodiamidate morpholino oligomers ("PMOs") to induce exon skipping in the DMD gene. Popplewell et al., Mol. Ther. 17(3):554-61 (2009) ("Popplewell 2009"). A set of 66 PMOs was designed to target exons 44-46, 51 and 53. *Id.* at 554. To design the AONs, three algorithms were used to identify exonic splicing enhancers (ESEs) and exonic splicing suppressors or silencers, and a hybridization array analysis was performed. *Id.* at 554-55, Figure 1. "The coincidence of ESEs as predicted by two or more algorithms and hybridization peaks determined experimentally, was used to design arrays of 25mer and, subsequently 30mer PMOs." *Id.* For exon 53, 17 25mer PMOs and 6 30mer PMOs were designed. *Id.* at Figure 1f.

40. The exon-skipping activity of the exon 53 PMOs was determined by transfecting normal human skeletal muscle cells ("hSkMCs") with each PMO "hybridized to phosphorothioate-capped oligodeoxynucleotide leashes" at concentrations from 50 to 500 nM for 4 hours. *Id.* at 560. RNA was extracted 24 hours after transfection and the percentage of exon skipping was determined using RT-PCR, which is a semi-quantitative analysis. *Id.* The PMOs' annealing coordinates and % skipping at the reported "optimal concentration" are shown below in Table 3.

Table 3. Exon skipping results reported in Popplewell 2009

PMO	Annealing Coordinates	% Skipping (nM)		PMO	Annealing Coordinates	% Skipping (nM)
h53A1	H53A(+35+59)	12.7% (100)		h53D1	H53A(+149+173)	0% (500)
h53A2	H53A(+38+62)	9.7% (100)		h53D2	H53A(+158+183)	0.1% (500)
h53A3	H53A(+41+65)	2% (500)		h53D3	H53A(+170+194)	3.7% (500)
h53A4	H53A(+44+68)	10.5% (500)		h53D4	H53A(+182+206)	12.3% (500)
h53A5	H53A(+47+71)	9% (250)		h53D5	H53A(+188+212)	7.9% (500)
h53A6	H53A(+50+74)	0.3% (500)		h53A30/1	H53A(+30+59)	52.4% (100)
h53B1	H53A(+69+93)	0% (500)		h53A30/2	H53A(+33+62)	87.2% (100)
h53B2	H53A(+80+104)	0.6% (500)		h53A30/3	H53A(+34+65)	80.1% (250)
h53B3	H53A(+90+114)	3% (500)		h53A30/4	H53A(+39+68)	38.6% (100)
h53C1	H53A(+109+133)	0% (500)		h53A30/5	H53A(+42+72)	9.4% (100)
h53C2	H53A(+116+140)	0% (500)		h53A30/6	H53A(+45+74)	35.9% (500)
h53C3	H53A(+128+152)	0% (500)				

Id. at Table S1.

41. These results further demonstrate the unpredictability of designing AONs to induce exon 53 skipping. First, despite the publication of Wilton PCT '057, the Dickson research group undertook the effort and expense of synthesizing and testing AONs covering a significant portion of exon 53 – starting at +30 and continuing through +212. The AONs selected by the Dickson group show that they did not consider the +23 to +69 region of exon 53 to be of particular interest, much less a “hot spot.” Further, the AON selection shows that researchers in the field did not view the Wilton laboratory’s previously published results as

identifying the boundaries of a “hot spot” on either end. Typically, when refining AON design, researchers would create AONs that extended slightly beyond a previously-identified active region. In this case, the Dickson group’s design of AONs starting at +30 indicates that they considered the amenable region to start around +30 or a few bases further into exon 53. And, the design and testing of numerous AONs extending beyond +69 of exon 53 indicates they did not view this base as the end of the “hot spot.”

42. Popplewell’s results also confirmed Wilton 2007’s observation that longer AONs worked better for exon 53. The 30mer AONs targeted a region of exon 53 spanning +29 to +73. *Id.* These AONs induced skipping between 9.4% to 87.2%. *Id.* For each of h53A1-h53A6, a corresponding 30mer that has an additional 5 bases on the 5’ end was tested. When compared to the 25mer AONs with overlapping sequences, the 30mers showed improved exon skipping. *See id.* However, it cannot be discerned whether that improvement is due to the length of the AON or the choice of annealing site. No other 30mer AONs targeting other regions of exon 53 were tested.

43. Popplewell’s results also confirm that even AONs that bind to overlapping annealing sites may have significant variability in the amount of skipping induced. For example, H53A(+47+71) induced 9% exon skipping at 250 nM. *Id.* Shifting the AON three bases 3’ to H53A(+50+74) essentially abolished all exon skipping, even though both AONs were the same length. *Id.* Similarly, H53A(+42+72) induced 9.4% exon skipping at 100 nM, however shifting the annealing site three bases upstream dramatically improved skipping at that concentration, while shifting the annealing site three bases downstream also improved skipping, although to a lesser degree. *Id.* These effects were observed even though all three AONs were 30mers. *Id.*

Table 4. Summary of results reported in Popplewell 2009.

PMO	Annealing Coordinates	% Skipping		PMO	Annealing Coordinates	% Skipping
h53A1	H53A(+35+59)	12.7%		h53A30/1	H53A(+30+59)	52.4%
h53A2	H53A(+38+62)	9.7%		h53A30/2	H53A(+33+62)	87.2%
h53A3	H53A(+41+65)	2%		h53A30/3	H53A(+34+65)	80.1%
h53A4	H53A(+44+68)	10.5%		h53A30/4	H53A(+39+68)	38.6%
h53A5	H53A(+47+71)	9%		h53A30/5	H53A(+42+72)	9.4%
h53A6	H53A(+50+74)	0.3%		h53A30/6	H53A(+45+74)	35.9%

d. Popplewell (Royal Holloway): January 2010

44. In January 2010, the Dickson group published another paper reporting the testing of AONs for skipping exon 53. Popplewell et al., *Neuromuscular Disorders*. 20(2):102-110 (2010) (“Popplewell 2010”). Popplewell 2010 includes co-authors outside of the Dickson group, including Steve Wilton and Annemieke Aartsma-Rus. However, the AON design and testing was done by the Dickson group researchers. The 24 PMOs described in Popplewell 2010 were tested in primary human myoblasts from a DMD patient because the hSkMCs used in Popplewell 2009 do not “allow assessment of the therapeutic effect at the protein level (*i.e.* dystrophin restoration”) because they are wild-type cells from healthy subjects carrying no mutation. *Id.* at 104. The primary human myoblasts were derived from skeletal muscle biopsy samples from a DMD patient with a deletion of exons 45-52. *Id.* at 103. The “most bioactive” AON identified by Wilton—H53A(+39+69)—was also synthesized as a PMO and tested as a comparator. *Id.* at 107. Popplewell 2010 used a different naming nomenclature for AONs than Popplewell 2009. Table 5 below provides a list of the PMOs tested in Popplewell 2010 and the corresponding PMO in Popplewell 2009.

Table 5. Summary of results reported in Popplewell 2010.

Popplewell 2010 PMO name	Popplewell 2009 name	Annealing Coordinates	Popplewell 2010 PMO name	Popplewell 2009 name	Annealing Coordinates
PMO-A	h53A1	H53A(+35+59)	PMO-T	h53D1	H53A(+149+173)
PMO-B	h53A2	H53A(+38+62)	PMO-U	h53D2	H53A(+158+183)
PMO-C	h53A3	H53A(+41+65)	PMO-V	h53D3	H53A(+170+194)
PMO-D	h53A4	H53A(+44+68)	PMO-W	h53D4	H53A(+182+206)
PMO-E	h53A5	H53A(+47+71)	PMO-X	h53D5	H53A(+188+212)
PMO-F	h53A6	H53A(+50+74)	PMO-G	h53A30/1	H53A(+30+59)
PMO-N	h53B1	H53A(+69+93)	PMO-H	h53A30/2	H53A(+33+62)
PMO-O	h53B2	H53A(+80+104)	PMO-I	h53A30/3	H53A(+34+65)
PMO-P	h53B3	H53A(+90+114)	PMO-J	h53A30/4	H53A(+39+68)
PMO-Q	h53C1	H53A(+109+133)	PMO-K	h53A30/5	H53A(+42+72)
PMO-R	h53C2	H53A(+116+140)	PMO-L	h53A30/6	H53A(+45+74)
PMO-S	h53C3	H53A(+128+152)	PMO-M	N/A	H53A(+39+69)

Id. at Table 1.

45. Popplewell 2010 states that +29 to +74 of exon 53 was “the region previously shown to be in open conformation, binding to which interferes with spliceosome-mediated pre-mRNA splicing, such that exon 53 is skipped,” citing to Popplewell 2009 and Wilton 2007 for this premise. *Id.* at 104. As a preliminary matter, I am unaware that such an “open conformation” region was “shown,” *i.e.*, assessed, measured or defined by the Dickson group or Wilton group. It was a hypothesis that was never proven. Further, the basis for this statement is not found in either Popplewell 2009 or Wilton 2007. Popplewell 2009 suggests areas of “open conformation” based on a hybridization array and MFOLD analysis, but not the +29 to +74 region.⁴ Popplewell 2009 at Fig. 1(d) and Fig. 2. Wilton 2007 neither discusses any “open

⁴ The MFold program has weaknesses that are and were well known at the time, namely, the RNA structure predicted is not guaranteed to be the native structure, MFold ignores complex RNA structures such as pseudoknots, and finally and most importantly, pre-RNA molecules do not exist in isolation, and nor do they exist as short RNAs that are the subject of MFold analysis.

conformations” nor the +29 to +74 region of exon 53. Indeed, Wilton 2007 states “[n]o single motif emerged as a reliable AO target to bring about consistent exon removal.” Wilton 2007 at 1293. As discussed above, the only exon 53 AON discussed in Wilton 2007 is H53A(+39+69). Popplewell 2009 concludes “our findings show that no single design tool is likely to be sufficient in isolation to allow the design of a bioactive AO, and empirical analysis is still required.” Popplewell 2009 at 559.

46. Popplewell 2010 directly compared “13 PMOs, whose target sites are within the sequence +29 to +74 of exon 53... at a 300 nM dose by nucleofection” in primary human patient myoblasts, culturing the cells for 3-21 days before RNA or protein was extracted to determine whether the PMO induced skipping of exon 53.⁵ *Id.* at 103. Because nucleofection was used, leashes were not necessary to facilitate delivery. *Id.* I note that there are differences between the assays described in Popplewell 2010 and Popplewell 2009 and Wilton PCT '057 that make it difficult to draw a general conclusion from these three studies, including AON backbone, AON length, cells used, incubation conditions, and transfection methods.

47. The results are shown in Table 6 below.⁶

Table 6. Summary of results reported in Popplewell 2010.

PMO	Annealing Coordinates	% Skipping		PMO	Annealing Coordinates	% Skipping
PMO-A	H53A(+35+59)	68%		PMO-G	H53A(+30+59)	73%
PMO-B	H53A(+38+62)	56%		PMO-H	H53A(+33+62)	68%
PMO-C	H53A(+41+65)	15%-26%		PMO-I	H53A(+34+65)	63%
PMO-D	H53A(+44+68)	15%-26%		PMO-J	H53A(+39+68)	37%
PMO-E	H53A(+47+71)	15%-26%		PMO-K	H53A(+42+72)	15%-26%
PMO-F	H53A(+50+74)	15%-26%		PMO-L	H53A(+45+74)	15%-26%
				PMO-M	H53A(+39+69)	52%

⁵ However, Popplewell 2010 does not report results for an AON that includes +29.

⁶ The exact percentage of skipping for PMO-C, -D, -E, -F, -K, and -L were not reported; rather only that these were the least effective of the 13 tested and produced skipping “between 15% and 26%.” Popplewell 2010 at 104.

48. PMOs -A, -B, -G, -H, -I, and -M were selected for dose-response and time course experiments because they were the most effective. *Id.* PMO-G “gave sustained high levels of exon skipping (over 60%) for the 21 days” but PMO-A and PMO-H “also appear to perform very well.” *Id.* PMOs -A, -G, -H, -I, and -M were then transfected into humanized mice, RNA was extracted from muscles and analyzed for exon 53 skipping. *Id.* at 107. The results are shown in Table 7 below.

Table 7. Summary of results reported in Popplewell 2010.

PMO	Annealing Coordinates	% Skipping
PMO-A	H53A(+35+59)	8%
PMO-G	H53A(+30+59)	7.2%
PMO-H	H53A(+33+62)	4.8%
PMO-I	H53A(+34+65)	7.6%
PMO-M	H53A(+39+69)	<1% (limit of detection)

49. I note that PMO-M, which is reported as having essentially undetectable skipping, has the same annealing site coordinates, (+39+69), as the AON that Wilton PCT '057 reported as being a strong skipper in 2'-O-methyl form. Again, due to the lack of information in Wilton PCT '057 regarding experimental conditions, it is not possible to discern whether this dramatic difference in skipping activity is due to variations in experimental parameters or backbone chemistry. *See* Dowdy Rpt. ¶ 645 (opining that “a POSA could not draw meaningful conclusions as to the relative skipping efficiencies of ASOs... versus target regions reported in the prior art” due to differences including chemical backbones, transfection conditions, and ASO concentrations.”). Regardless, Popplewell 2010’s results with PMO-M contradict Dr. Dowdy’s claim that a “hot spot” within exon 53 exists, much less between the +27 and +69 bases of exon 53.

50. Popplewell recommends PMOs targeting the +30 to +65 region of exon 53 for consideration for clinical development. Popplewell 2010 at 109. The authors note that “although there are tools available to aid the design of AOs for the targeted skipping of DMD exons, the empirical analysis of AOs is still required.” *Id.* at 108. They stated that the data presented indicates that “PMOs targeting within the sequence of +30+65 of exon 53 (namely PMO-A, -G, and -H) produce levels of exon skipping that may be considered effective” but the “possibility that a stepped base-by-base screening of AOs across the entirety of exon 53 and some indeterminate distance into the flanking intronic sequences might reveal an AO with a better dose-response and longevity of action profile.” *Id.* Thus, Popplewell 2010 authors, including Dr. Wilton, recognized that a base-by-base screen of the *entire* exon could identify AONs that might be more effective than the existing AONs in the +30 to +65 region. Thus, a POSA would understand that Popplewell 2010 was not describing +30 to +65 as a “hot spot.”

4. Sazani and Kole (Sarepta Therapeutics): April 2010

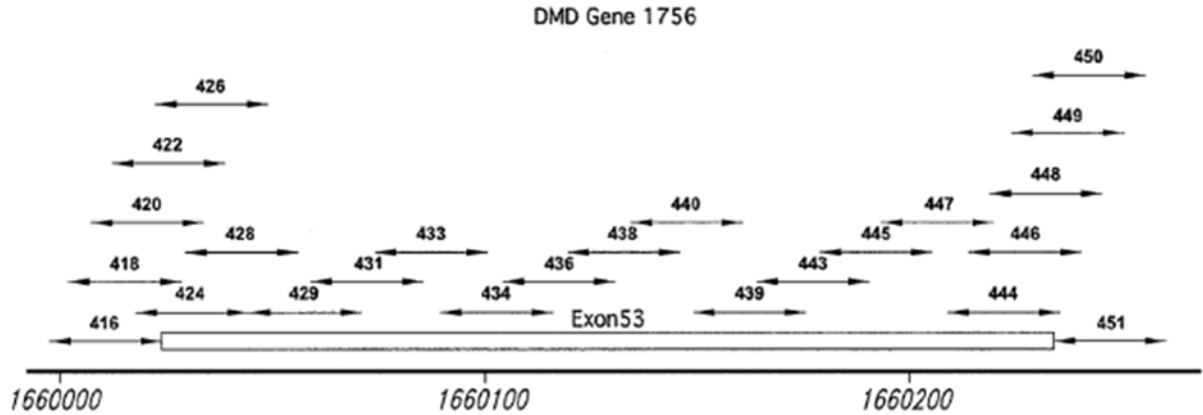
51. PCT Patent Publication No. WO2010/048586 by Peter Sazani and Ryszard Kole of AVI BioPharma (which later changed its name to Sarepta Therapeutics) (“Sazani ’586”) published on April 29, 2010. This publication disclosed peptide conjugated-PMOs (“PPMOs”) targeting exons 44-55 of the dystrophin gene. Sazani ’586 at 69:24-25; 79-103. A series of 24 overlapping PPMOs targeting exon 53 were designed and tested for skipping efficacy in human rhabdomyosarcoma (“RD”) cells as shown in Table 8 below. *Id.* at 79-103. An additional forty-four AONs targeting exon 53 are also disclosed—twenty 30mers (SEQ ID Nos: 417, 419, 421, 432, 425, 427, 430, 432, 435, 437, 441, 442, and 626-633) and twenty-four 20mers (SEQ ID Nos: 452-451). *Id.* at 79-103.

Table 8. PPMOs made and tested in Sazani '586

SEQ ID NO.	PPMO Name	Annealing Coordinates
416	Hu.DMD.Exon53.25.001	H53A(-26-1)
418	Hu.DMD.Exon53.25.002.2	H53A(-20+5)
420	Hu.DMD.Exon53.25.003.02	H53A(-15+10)
422	Hu.DMD.Exon53.25.004.02	H53A(-10+15)
424	Hu.DMD.Exon53.25.005.02	H53A(-5+20)
426	Hu.DMD.Exon53.25.006.02	H53A(+1+25)
428	Hu.DMD.Exon53.25.008	H53A(+8+32)
429	Hu.DMD.Exon53.25.008.02	H53A(+23+47)
431	Hu.DMD.Exon53.25.009.02	H53A(+38+62)
433	Hu.DMD.Exon53.25.010.02	H53A(+53+77)
434	Hu.DMD.Exon53.25.011	H53A(+68+92)
436	Hu.DMD.Exon53.25.012.02	H53A(+83+107)
438	Hu.DMD.Exon53.25.013.02	H53A(+98+122)
439	Hu.DMD.Exon53.25.014	H53A(+128+152)
440	Hu.DMD.Exon53.25.014.02	H53A(+113+137)
443	Hu.DMD.Exon53.25.016.02	H53A(+143+167)
444	Hu.DMD.Exon53.25.017	H53A(+188+212)
445	Hu.DMD.Exon53.25.017.02	H53A(+158+182)
446	Hu.DMD.Exon53.25.018	H53D(+20-5)
447	Hu.DMD.Exon53.25.018.02	H53A(+173+197)
448	Hu.DMD.Exon53.25.019	H53D(+15-10)
449	Hu.DMD.Exon53.25.020	H53D(+10-15)
450	Hu.DMD.Exon53.25.021	H53D(+20-5)
451	Hu.DMD.Exon53.25.022	H53D(-1-25)

52. For a graphical illustration of the exon 53 oligos from Sazani '586, I reproduce Figure 4A, below:

Dystrophin Exon 53 Scan Oligos



53. In my opinion, it is noteworthy that the Sarepta researchers undertook the cost and expense of synthesizing and testing AONs that spanned the entirety of the 212 base human exon 53, plus 25 bases upstream and downstream of the exon, for skipping activity. In my opinion, this shows that there was no recognized “hot spot” within exon 53 at that time. *Cf.* Dowdy Rpt. ¶¶ 99, 104. Indeed, it appears that the Sarepta researchers either did not rely on Wilton PCT ‘057 at all to design AONs for the initial screen or were more focused outside of the +39+69 region that the Wilton laboratory had identified as an amenable region in Harding 2007.

54. In Sazani ’586, human RD cells were treated with each of the 24 PPMOs and after 48 hours RNA was extracted and examined for exon skipping using RT-PCR. *Id.* at 69-73. Although Sazani ’586 reports that the PPMOs were evaluated at “various concentrations as described above,” the actual concentrations tested for each PPMO are not disclosed.⁷ After testing all twenty-four PPMOs, three of them, H53A(+8+32), H53A(+23+47), and

⁷ The materials and methods section merely states that the “PPMOs were diluted in warmed media to the desired molarity; cells were treated in a total of 1.0mL PPMO per well.” Sazani ’586 at 69:28-70:1.

H53A(+38+62), “were identified as effective in inducing exon-skipping and selected for additional evaluation” *Id.* at 75:30-76:1. Sazani ’586 conducted a dose-ranging study on the selected exon 53 PPMOs, testing at 1.0, 2.0, 3.0, 5.0 and 10.0 uM. Fig. 4C-4G. After further testing of H53A(+8+32), H53A(+23+47), and H53A(+38+62), H53A(+23+47) was shown to be the most effective. *Id.* at 76:2-4.

55. Dr. Dowdy concludes that these results “independently confirmed the hot spot identified by Dr. Wilton and his coinventors [in Wilton PCT ’057], including in particular the region targeted by H53A(+23+47).” Dowdy Rpt. ¶ 104. I disagree. Dr. Dowdy’s conclusions overstate the disclosures of Sazani ’586. Importantly, the results of the preliminary screen Sazani relied on to select the three AONs for additional evaluation are not disclosed in the specification, and thus cannot be evaluated. Dr. Dowdy appears to assume that the remaining twenty-one AOs were not “effective.” This assumption is clearly unsupported, substantively or logically, and complicated by the fact that Sazani ’586 does not define “effective.”

56. For example, if what the Wilton PCT ’057 discloses as “very faint skipping” was not considered to be “effective” in the Sazani ’586 screen, there would be no report of such results in Sazani ’586. Accordingly, no conclusions can be made as to whether any particular region is a “hot spot” from this data.

57. Sazani ’586 compares PPMO H53A(+23+47) to PPMO H53A(+39+69), the most active AON sequence identified by Wilton PCT ’057 and Harding 2007 (as 2’-O-methyl). I note that this PPMO version of H53A(+39+69) should have ensured very highly effective delivery into the RD cells. In Sazani ’586, H53A(+23+47) “was shown to be superior to” H53A(+39+69). Sazani ’586 at 76:15-16 and Fig. 4H. Indeed, in Figure 4H, H53A(+39+69) shows no discernible skipping activity whatsoever in RD cells. *Id.* This is directly contradictory

to the results reported in Wilton PCT '057 where the 2'-O-methyl version of H53A(+23+47) was reported to induce "very faint skipping to 50 nM" while H53A(+39+69) was reported to induce "strong skipping to 50 nM." Wilton PCT '057 at Table 39; *see also* Harding 2007 at Fig. 4; Wilton 2007 at Fig. 2. Thus, rather than "further confirming" the existence of a "hot spot" as Dr. Dowdy opines, Sazani '586 in fact supports the opposite conclusion. *Cf.* Dowdy Rpt. ¶¶ 103-104.

58. This discrepancy highlights the variability of exon skipping assays across different laboratories, and different experiments, as well as possibly the effects of different AON chemistries. These results, which truly are contradictory, exemplify why it was essential for those working in the field to independently confirm results rather than relying solely on published data from other laboratories. The experimental conditions for testing AONs typically yielded variable results, which made identifying an AON that was likely to perform well in human subjects a very challenging undertaking.

59. I note that Dr. Dowdy's analysis of the disclosures of Wilton PCT '057 is inconsistent with his analysis of the results reported in Sazani '586. According to Dr. Dowdy, Wilton PCT '057 defines a "hot spot" based on the far end points of overlapping AONs that had any degree of skipping activity, even faint. Dowdy Rpt. ¶ 99. Yet, even though Sazani '586 discloses that H53A(+8+32) is "effective," Dr. Dowdy does not extend the "hot spot" to include the end point (+8) of this AON – even though it overlaps with the other two AONs described as "effective." Instead, Dr. Dowdy concludes that the inventors of Sazani '586 "further confirmed the exon 53 hot spot." Dowdy Rpt. ¶ 103. If Dr. Dowdy were applying consistent criteria, he would have concluded that Sazani '586 expanded the hot spot to +8.

5. Wilton PCT '350: May 2011

60. PCT Patent Publication No. WO2011/057350 by Wilton et al., (“Wilton PCT '350”) published on May 19, 2011. This publication “relates to novel antisense compounds and compositions suitable for facilitating exon skipping.” Wilton PCT '350 at 1:4-5. The inventors state that, even in 2010, when this application was filed, that “[a]ttempts ... to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense compounds has been the result of empirical studies.” *Id.* at 33:12-16. 2'-O-methyl AONs were designed to target “known or predicted motifs or regions involved in splicing.” *Id.* at 33:23-26. The 2'-O-methyl AONs were synthesized and transfected into normal primary myoblasts. *Id.* at 34:5-10. After 24 hours, RNA was extracted and analysis of exon skipping was conducted using RT-PCR. *Id.* at 34:11-14

61. Twenty-five 2'-O-methyl AONs targeting exon 53 were designed and synthesized. *Id.* at Table 43. These AONs span approximately the first half of exon 53 from H53A(-49-26) (an AON targeting intronic sequences near the exon 53 splice acceptor site) to H53A(+69+98). *Id.* Thus, like Sazani '586, the Wilton laboratory spent time and resources synthesizing and testing AONs targeting portions of exon 53 for exon-skipping activity that were both outside of and within the “hot spot” Dr. Dowdy asserts they identified in 2005. Notably, none of the AONs disclosed in Wilton PCT '350 targeted coordinates upstream of +27 in exon 53; none covered the 5' end of the alleged “hot spot” at +23. In my opinion, this shows the Wilton laboratory did not regard the region of +23 to +27 to be part of a “hot spot” or even worth further testing.

62. Like in Wilton PCT '057, the results are reported as “skipping to” or “skipping at” a certain concentration. *Id.* at Table 43. The results are reproduced in Table 9 below.

Table 9. Summary of results reported in Wilton PCT '350.

AON	Ability to Induce Skipping
H53A(-15+15)	No skipping
H53A(-32-06)	No skipping
H53A(-38-13)	No skipping
H53A(-49-26)	No skipping
Hint52(-47-23)	No skipping
H53A(+27+56)	Strong skipping to 25 nM, faint at 5 nM
H53A(+27+59)	Strong skipping to 10 nM, faint at 5 nM
H53A(+30+59)	Not reported
H53A(+30+64)	Strong skipping to 25 nM, faint at 10 nM
H53A(+30+69)	Strong skipping to 25 nM, faint at 5 nM
H53A(+33+63)	Strong skipping to 25 nM, faint at 5 nM
H53A(+33+65)	Strong skipping to 25 nM, faint at 2.5 nM
H53A(+33+67)	Strong skipping to 50 nM, faint at 5 nM
H53A(+35+67)	Strong skipping to 25 nM
H53A(+36+70)	Reasonable skipping to 5 nM
H53A(+37+67)	Strong skipping to 25 nM
H53A(+39+65)	Skipping 50 nM
H53A(+39+67)	Skipping 100 nM
H53A(+39+69)SNP	Skipping to 25 nM
H53A(+39+71)	Strong skipping to 25 nM
H53A(+40+70)	Skipping 50 nM
H53A(+41+69)	Skipping 50 nM
H53A(+42+71)	Strong skipping to 100 nM, faint at 5 nM
H53A(+43+69)	Skipping 50 nM
H53A(+69+98)	Skipping at 50 nM

63. There is no definition of what was considered to be “skipping,” “strong skipping” or “faint” skipping. The only objective exon 53 skipping data provided is in Figure 43, for H53A(+33+65).

6. Watanabe et al.: August 2011

64. Watanabe et al. filed PCT/JP2011/070318 on August 31, 2011 (“Watanabe PCT ’318”). I understand the patents asserted by NS each claim priority to Watanabe PCT ’318, and share a common specification. For convenience, I will cite to the specification of U.S. 10,647,741 (“the ’741 patent”). I understand the inventors later published an article describing the work underlying Watanabe PCT ’318. Watanabe et al., *Mol Ther Nucleic Acids*, 13:442-449 (2018) (“Watanabe 2018”). As Dr. Dowdy acknowledges, the inventors of Watanabe PCT ’318 made and tested a series of overlapping 25 base AONs targeting the *entirety* of exon 53. ’741 patent, Table 7 and Figs. 13-17; Watanabe 2018, at 443; Dowdy Rpt. ¶¶ 459-460. That the Watanabe PCT ’318 inventors undertook the effort and cost of evaluating the entirety of exon 53 supports my opinion that the information known in the art at the time, including that disclosed in Wilton PCT ’057, was insufficient to identify or define a “hot spot” within exon 53.

B. Designing An AON That Would Induce Exon Skipping Was Unpredictable As of August 2011.

1. There Were No Recognized “Hot Spots” as of August 2011.

65. Dr. Dowdy concludes that “by August 31, 2011, the hot spot within exon 53 had been identified and repeatedly verified.” Dowdy Rpt. ¶ 110. I disagree—as described above, the conclusions that Dr. Dowdy drew from these publications were a misreading of the data as a whole. These laboratories use a variety of AON chemistries (e.g., 2’-O-methyl, PMOs, PPMOs, and ENAs) and divergent (or undisclosed) experimental parameters. Even the concept of a “hot spot” ignores the impact of length on an AON’s ability to induce exon skipping, which is an important factor linked to the fundamental biophysical properties of the AON and its binding affinity, e.g., how strongly it is likely to bind to the target sequence. Further, overarching

conclusions cannot be drawn from comparing these studies or in the field at all—as is well-known amongst those with more extensive experience with exon skipping studies, as illustrated by the articles discussed above.

66. Dr. Dowdy provides a summary of the AONs published as of August 31, 2011 in support of his conclusion. Dowdy Rpt. Fig. 18. In my opinion, Dr. Dowdy's Figure 18 is a classic example of confirmation bias. First, Dr. Dowdy selectively included only those AONs that overlap with the exon 53 region he believes was the "hot spot."

67. Second, Dr. Dowdy omits or ignores the information in the very same publications (and others) that show researchers designing and testing AONs outside of his putative "hot spot." For example, the Royal Holloway group reported on AONs both upstream and downstream of the "hot spot" (h53B1, h53B2, h53B3, h53C1, h53C2, h53C3, h53D1, h53D2, h53D3, h53D4, h53D5), as did Sarepta (SEQ ID Nos: 416, 418, 420, 422, 424, 426, 436, 436, 439, 440, 443, 444, 445, 446, 447, 448, 449, 450, 451 in Sazani '586), as did Wilton (H53A(-15+15), H53A(-32-06), H53A(-38-13), H53A(-49-26), Hint52(-47-23), H53A(+69+98) in Wilton PCT '350). Indeed, the majority of the AONs in each of the publications discussed above were outside of Dr. Dowdy's alleged "hot spot." If Wilton PCT '057 had identified a "hot spot" recognizable by other researchers, there would have been no need for NS, Popplewell or Sazani to screen the entirety of exon 53 in 2010, or for Wilton to make and test AONs outside of the "hot spot" in 2011.

68. Third, Figure 18 lacks nuance and important information. Dr. Dowdy failed to note the AONs that induce *little or no* exon skipping overlapping with his putative "hot spot." For example, the 2'-O-methyl versions of H53A(+23+47), H53A(+45+69) and H53A(+39+62) in Wilton PCT '057, h53A3 (+41+65), h53A5 (+47+71), h53A6 (+50+74), h53A30/5 (+42+72)

in Popplewell 2009, and the PPMO version of H53A(+39+69) in Sazani '586, all were reported to induce little or no skipping. Yet in Figure 18, Dr. Dowdy fails to distinguish between these AONs that had barely any detectable exon skipping (or yielded inconsistent results) and AONs that were reported to have greater activity. In my opinion, a POSA would take this additional information into account in designing AONs for exon 53 skipping or drawing any conclusions about the existence of a “hot spot.”

69. In order to determine whether there are one or more “hot spot(s)” within exon 53 that, if targeted with an AON, would induce exon skipping, a full micro-walk of the exon would be necessary. Such a “micro-walk” would require an AON of a single length be moved base-by-base along exon 53. It would be important to understand which AONs induce exon skipping, but also to understand which AONs do not induce exon skipping. Moreover, it would be necessary to repeat this micro-walk for several AON lengths to account for the impact the length has on the ability of an AON to induce skipping. These studies had not been completed by August 2011, and to my knowledge have still not been published. Even if such micro-walk studies were completed, there may not be a discrete “hot spot” within exon 53 because as I note in my Opening Report, moving an AON by even a single base in the 5’- or 3’-direction can change its ability to induce exon skipping, sometimes very significantly.

2. Sequence Does Not Predict Exon-Skipping Activity

70. Dr. Dowdy opines that “a POSA would have reasonably expected “that ASOs complementary to the region of H53A(+30+65) “would successfully cause exon skipping.” Dowdy Rpt. ¶ 418. I disagree. As I describe in my Opening Report, even today, one cannot predict *a priori* whether a given AON will induce exon skipping. Opening Report §§ IV.F-H; V.B-C. Dr. Wilton recognizes this, as he has repeatedly reported that the empirical studies are

necessary to identify AONs capable of inducing exon skipping. *E.g.*, Wilton PCT '057 at 35:3-6; Harding 2007; Wilton PCT '350 at 33:12-16; Aung-Htut et al., *Int. J. Mol. Sci.* 20:5030 (2019) (“Wilton 2019”), at 2 of 12.

71. As I describe above, and in my Opening Report, one cannot predict whether a given AON will induce exon skipping by virtue of its sequence or annealing site on the exon RNA. As was explained in Aartsma-Rus 2002, and was acknowledged in Popplewell 2009, the RNA's native structure may change the accessibility of a particular AON to bind to the exon pre-mRNA and thus induce exon skipping. Aartsma-Rus 2002 at S76; Popplewell 2009 at Figure 2. It is the native structure in the context of the intracellular nuclear compartment where the pre-mRNA resides. Moreover, this native structure is influenced not just by chemistry of the nuclear compartment but by a plethora of proteins that are present and that associate with the RNA. Knowing which sequences are accessible to AONs is not possible (yet) to predict because the overall three-dimensional structure of the entire dystrophin pre-mRNA has yet to be determined and thus the ability for any AON to bind to a given dystrophin pre-mRNA sequence cannot even be modeled, much less predicted.

72. As of 2011, primitive RNA structure prediction algorithms such as MFold were limited in usefulness, as discussed above. Further, dystrophin is a very large gene, and modelling it in its totality or within its native context in the nuclear compartment, with all the many other RNAs and components in this cellular compartment was not and is not possible. Models were and are incapable of taking into account the many chemicals and proteins that bind to RNA and alter its structure and accessibility. Therefore, the idea that one could define an open confirmation by modelling and predict likely target sequences, or the activity of particular sequences, was not and is not possible.

73. To predict therapeutically relevant levels of activity, this modeling would need to be done for many different cell types - such as skeletal muscle and heart muscle cells, and others. Further, many different types of mutations are relevant to skipping of exon 53, and how these mutations, many of which are large deletions, influence pre-mRNA structure and accessibility has not even begun to be studied.

74. The end result of this complexity is the need for empirical studies to identify AONs that are capable of skipping an exon, and the lack of any “expectation of success” in identifying an AON that induces exon skipping by merely designing an AON to anneal within a given region of exon 53 without testing that AON empirically. I note that a region such as the +30 to +65 recommended by Popplewell 2010, there are many millions of potential AONs that vary in length, chemistry, and other structural features that affect activity.

3. Dr. Wilton and Sarepta Have Agreed That Exon Skipping Is Unpredictable

75. Exon skipping was unpredictable as of 2011 and remains unpredictable today. For example, in 2019, Dr. Wilton stated “[u]ltimately, identifying target domains within a pre-mRNA that influence splicing and then refining AO design through micro-walking must be done empirically.” Wilton 2019 at 2 of 12. Dr. Wilton describes “general guidelines that are efficient and effective in developing biologically active “AONs, with steps that include “further refine[ment] by micro-walking around the annealing site and/or altering AO length” and “transfection studies over a range of concentrations.” *Id.* Dr. Wilton also wrote “experimental optimisation is therefore critical to the development of AO therapeutics.” *Id.* at p. 8 of 12.

76. In 2016, Sarepta filed US patent application number 14/776,533 (the “’533 Application”), which claimed AONs targeting exon 53 for exon skipping. ’533 Application at

claims 1-20. I understand from counsel that the '533 Application claimed priority to a 2013 provisional application and thus the relevant date of invention is the filing date of that provisional application, March 14, 2013. *Id.* at 6-9.

77. During prosecution of the '533 Application, Sarepta argued that there is a “high level of unpredictability” in the art with “no reasonable expectation of success” in “selecting specific [AONs] to induce effective exon skipping” of exon 53. '533 Application 8-28-2017 Office Action Response at 8, 20-21. Rather Sarepta acknowledges that “each [AON] must be individually made and tested, the results are *unpredictable* as to whether any exon skipping . . . will be achieved.” *Id.* at 15. Even AONs that “overlap the same region” may not uniformly induce exon skipping. *Id.* Sarepta further acknowledges that the effect of the chemistry (*i.e.* 2'-O-methyl or PMO) of an [AON] on its efficiency of exon skipping is unpredictable.” *Id.*

78. In a subsequent office action response, Sarepta argued that “a few changes to the [AON] can provide great changes in exon skipping efficiency.” '533 Application 12-14-2018 Office Action Response at 22.

79. Thus, as Sarepta has acknowledged, even as of 2013 one could not predict whether any given AON could induce exon skipping. Accordingly, there was no expectation of success in arriving at an AON that would induce exon skipping by merely selecting a sequence that would bind within a given region of the dystrophin pre-mRNA.

V. RESERVATION OF RIGHTS AND TRIAL EXHIBITS

80. I reserve the right to amend or supplement my opinions once I review any new/additional information and/or other new/additional documents or information subsequently produced by Sarepta, UWA, or any other party, including via rebuttal expert reports of Sarepta's and UWA's experts.

81. At trial I may use visual aids and demonstratives to show the bases for my opinions, such as photographs, drawings, excerpts from documents and materials I considered, videos, and animations. I reserve the right to utilize these at trial.